**Basic Information**

- **Catalog Number:** 16534-1-AP
- **Size:** 150μl, Concentration: 500 μg/ml by Nanodrop and 307 μg/ml by Bradford method using BSA as the standard.
- **Source:** Rabbit
- **Isotype:** IgG
- **Immunogen Catalog Number:** AG9812
- **GenBank Accession Number:** BC014265
- **GeneID (NCBI):** 115908
- **Full Name:** collagen triple helix repeat containing 1
- **Calculated MW:** 243 aa, 26 kDa
- **Observed MW:** 30 kDa
- **Purification Method:** Antigen affinity purification
- **Recommended Dilutions:**
  - WB: 1:500-1:2000
  - IP: 0.5-4.0 ug for IP and 1:500-1:1000 for WB
  - IHC: 1:20-1:200
  - IF: 1:50-1:500

**Applications**

- **Tested Applications:** IF, IHC, IP, WB, ELISA
- **Cited Applications:** IF, IHC, WB
- **Species Specificity:** human, mouse, rat
- **Cited Species:** human, rat

**Note-IHC:** suggested antigen retrieval with **TE buffer pH 9.0**; (*) Alternatively, antigen retrieval may be performed with **citrate buffer pH 6.0**

**Background Information**

Collagen triple helix repeat-containing protein 1 (CTHRC1), also known protein NMTC1, is a glycosylated secreted protein. CTHRC1 was identified as a novel molecular in injured and diseased arteries where it may contribute to vascular remodeling by limiting collagen matrix deposition and promoting cell migration (PMID: 15618538). It has been reported that CTHRC1 is a positive regulator of osteoblastic bone formation (PMID: 18779865). Besides, studies reveal that CTHRC1 expression is implicated in various malignancies, including hepatocellular carcinoma, gastric carcinoma, colon cancer, and lung cancer (PMID: 23922981; 24504172; 24746208; 25139095).

**Notable Publications**

| Author          | Pubmed ID | Journal              | Application |
|-----------------|-----------|----------------------|-------------|
| Huifang Zhou    | 31576160  | Onco Targets Ther    | IHC         |
| Biying Guo      | 29021002  | J Ovarian Res        | WB, IHC     |
| Lu-Ying Li      | 31119444  | Clin Exp Metastasis  | WB          |

**Storage**

- **Storage:** Store at -20°C. Stable for one year after shipment.
- **Storage Buffer:** PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
- **Aliquoting is unnecessary for -20°C storage**

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For technical support and original validation data for this product please contact:

T: 1 (888) 4PTGLAB (1-888-478-4522) [toll free in USA], or 1(312) 455-8498 (outside USA)

E: proteintech@ptglab.com

W: ptglab.com

This product is exclusively available under Proteintech Group brand and is not available to purchase from any other manufacturer.
A375 cells were subjected to SDS PAGE followed by western blot with 16534-1-AP (CTHRC1 antibody) at dilution of 1:800 incubated at room temperature for 1.5 hours.

Immunohistochemical analysis of paraffin-embedded human breast cancer using 16534-1-AP (CTHRC1 antibody) at dilution of 1:50 (under 10x lens).

Immunohistochemical analysis of paraffin-embedded human breast cancer using 16534-1-AP (CTHRC1 antibody) at dilution of 1:50 (under 40x lens).

IP Result of anti-CTHRC1 (IP:16534-1-AP, 3ug; Detection:16534-1-AP 1:500) with A375 cells lysate 3600ug.

Immunofluorescent analysis of (-20°C Ethanol) fixed A375 cells using 16534-1-AP (CTHRC1 antibody) at dilution of 1:50 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).
GAPDH (14C10) Rabbit mAb

For Research Use Only. Not For Use In Diagnostic Procedures.

| Applications: | Reactivity: | Sensitivity: | MW (kDa): | Source/Isotype: | UniProt ID: | Entrez-Gene Id: |
|---------------|-------------|--------------|----------|----------------|------------|----------------|
| WB, IHC-P, IF-IC, FC-FP | H M R Mk B Pg | Endogenous | 37 | Rabbit | P04406 | 2597 |

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity

GAPDH (14C10) Rabbit mAb detects endogenous levels of total GAPDH protein.

Species Reactivity:

Human, Mouse, Rat, Monkey, Bovine, Pig

Species predicted to react based on 100% sequence homology:

Pig

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide near the carboxy terminus of human GAPDH.

Background

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis. Though differentially expressed from tissue to tissue (1), GAPDH is thought to be a constitutively expressed housekeeping protein. For this reason, GAPDH mRNA and protein levels are often measured as controls in experiments quantifying specific changes in expression of other targets. Recent work has elucidated roles for GAPDH in apoptosis (2), gene expression (3), and nuclear transport (4). GAPDH may also play a role in neurodegenerative pathologies such as Huntington and Alzheimer’s diseases (4,5).

1. Barber, R.D. et al. (2005) Physiol. Genomics 21, 389-95.
2. Hara, M.R. and Snyder, S.H. (2006) Cell Mol. Neurobiol. 26, 527-38.
3. Zheng, L. et al. (2003) Cell 114, 255-66.
4. Bae, B.I. et al. (2006) Proc. Natl. Acad. Sci. USA 103, 3405-9.
5. Wang, Q. et al. (2005) FASEB J. 19, 869-71.

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

APPLICATIONS KEY

WB: Western Blotting  IHC-P: Immunohistochemistry  (Paraffin)  IF-IC: Immunofluorescence (Immunocytochemistry)  FC-FP: Flow Cytometry (Fixed/Permeabilized)

CROSS-REACTIVITY KEY

H: human  M: mouse  R: rat  Hm: hamster  Mk: monkey  Vr: virus  Mm: mink  C: chicken  Dm: D. melanogaster  X: Xenopus  Z: zebrafish  B: bovine  Dg: dog  Pg: pig  Sc: S. cerevisiae  Ce: C. elegans  Hr: horse  Rab: rabbit  All: all species expected

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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#2118

GAPDH (14C10) Rabbit mAb

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Overview

Product name: Anti-Id1 antibody

Description: Mouse polyclonal to Id1

Host species: Mouse

Tested applications:

Suitable for: WB, ICC/IF

Species reactivity:

Reacts with: Human

Predicted to work with: Mouse, Rat, Sheep, Cat, Dog, Pig, Chimpanzee, Rhesus monkey, Orangutan

Immunogen:
Full-length protein corresponding to amino acids 1-155 of Human Id1 (NP_002156.2).

Positive control:
Lysate from Id1 transfected 293T cells; HeLa cells

General notes:

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form: Liquid

Storage instructions:
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.

Storage buffer:
pH: 7.4
Constituent: 100% PBS

Purity:
Protein A purified

Clonality:
Polyclonal

Isotype:
IgG

Applications

6 References 2 Images
The Abpromise guarantee

The Abpromise guarantee covers the use of ab168256 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|-------------|-----------|-------|
| WB          |           | Use a concentration of 1 µg/ml. Predicted molecular weight: 16 kDa. |
| ICC/IF      |           | Use a concentration of 10 µg/ml. ab168256 must be purified for ICC/IF application. |

Target

Function: ID (inhibitor of DNA binding) HLH proteins lack a basic DNA-binding domain but are able to form heterodimers with other HLH proteins, thereby inhibiting DNA binding.

Sequence similarities: Contains 1 basic helix-loop-helix (bHLH) domain.

Developmental stage: Expression correlates with proliferation in some types of cells.

Cellular localization: Cytoplasm. Nucleus.

Images

All lanes: Anti-Id1 antibody (ab168256) at 1/500 dilution

Lane 1: ID1-transfected 293T cell lysate

Lane 2: Non-transfected 293T cell lysate

Lysates/proteins at 15 µl per lane.

Predicted band size: 16 kDa

Immunofluorescence analysis of paraformaldehyde-fixed HeLa cells, labeling ld1 using ab168256 at 10 µg/ml. Cells should be permeabilized by incubating for 15 minutes on ice with 2 mL of 0.1% Triton X-100 in PBS prior to staining.
Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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Product datasheet

Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker ab52635

RabMAb

★★★★★ 10 Abreviews  106 References  19 Images

Overview

Product name
Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker

Description
Rabbit monoclonal [EP1601Y] to Cytokeratin 5 - Cytoskeleton Marker

Host species
Rabbit

Specificity
Mouse reactivity is based on IHC (positive tissues: Liver, lung, brain and skin). However, WB was negative for Mouse brain, heart, kidney and spleen. There is background staining in mouse and rat islet.

Tested applications
Suitable for: Flow Cyt (Intra), ICC/IF, WB, IHC-P

Species reactivity
Reacts with: Mouse, Rat, Human

Immunogen
Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control
WB: A431 cell, human fetal skin, rat skin and mouse skin lysates. IHC-P: Squamous cell cervical, squamous cell lung and basal cell breast carcinoma tissue. Human transitional urinary bladder carcinoma tissue. Normal tonsil squamous, human cervical carcinoma, mouse skin and rat skin tissues. Flow Cyt (intra) and ICC/IF: A431 cells. ICC/IF: A431 cells.

General notes
This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production
For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Properties
Form: Liquid

Storage instructions: Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

Storage buffer:
- pH: 7.20
- Preservative: 0.01% Sodium azide
- Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified

Clonality: Monoclonal

Clone number: EP1601Y

Isotype: IgG

**Applications**

**The Abpromise guarantee**

Our Abpromise guarantee covers the use of ab52635 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application  | Abreviews | Notes                                      |
|--------------|-----------|--------------------------------------------|
| Flow Cyt (Intra) |           | 1/20.                                      |
| ICC/IF       |           | 1/100.                                     |
| WB           | ★★★★★ (2) | 1/10000. Detects a band of approximately 62 kDa (predicted molecular weight: 62 kDa). |
| IHC-P        | ★★★★★ (4) | 1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

**Target**

**Involvement in disease**

Defects in KRT5 are a cause of epidermolysis bullosa simplex Dowling-Meara type (DM-EBS) [MIM:131760]. DM-EBS is a severe form of intraepidermal epidermolysis bullosa characterized by generalized herpetiform blistering, milia formation, dystrophic nails, and mucous membrane involvement.

Defects in KRT5 are the cause of epidermolysis bullosa simplex with migratory circinate erythema (EBSMCE) [MIM:609352]. EBSMCE is a form of intraepidermal epidermolysis bullosa characterized by unusual migratory circinate erythema. Skin lesions appear from birth primarily on the hands, feet, and legs but spare nails, ocular epithelia and mucosae. Lesions heal with brown pigmentation but no scarring. Electron microscopy findings are distinct from those seen in the DM-EBS, with no evidence of tonofilament clumping.

Defects in KRT5 are a cause of epidermolysis bullosa simplex Weber-Cockayne type (WC-EBS) [MIM:131800]. WC-EBS is a form of intraepidermal epidermolysis bullosa characterized by blistering limited to palmar and plantar areas of the skin.

Defects in KRT5 are a cause of epidermolysis bullosa simplex Koebner type (K-EBS) [MIM:131900]. K-EBS is a form of intraepidermal epidermolysis bullosa characterized by generalized skin blistering. The phenotype is not fundamentally distinct from the Dowling-Meara type, although it is less severe.

Defects in KRT5 are the cause of epidermolysis bullosa simplex with mottled pigmentation (MP-
EBS) [MIM:131960]. MP-EBS is a form of intraepidermal epidermolysis bullosa characterized by blistering at acral sites and ‘mottled’ pigmentation of the trunk and proximal extremities with hyper- and hypopigmentation macules.

Defects in KRT5 are the cause of Dowling-Degos disease (DDD) [MIM:179850]; also known as Dowling-Degos-Kitamura disease or reticulate acropigmentation of Kitamura. DDD is an autosomal dominant genodermatosis. Affected individuals develop a postpubertal reticulate hyperpigmentation that is progressive and disfiguring, and small hyperkeratotic dark brown papules that affect mainly the flexures and great skin folds. Patients usually show no abnormalities of the hair or nails.

**Sequence similarities**

Belongs to the intermediate filament family.

**Images**

All lanes: Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635) at 1/1000 dilution

Lane 1: Human skin lysates prepared in RIPA lysis method
Lane 2: Human skin lysates prepared in 1%SDS Hot lysis method
Lane 3: Mouse skin lysates prepared in RIPA lysis method
Lane 4: Mouse skin lysates prepared in 1%SDS Hot lysis method

Lysates/proteins at 20 µg per lane.

**Secondary**

All lanes: Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

**Predicted band size:** 62 kDa

The lysates were prepared in 1%SDS Hot lysis method.

Observed MW: 62kDa

Blocking/diluting buffer and concentration: 5% NFDM/TBST
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue sections labeling Cytokeratin 5 with Purified ab52635 at 1:200 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Immunocytochemistry/Immunofluorescence analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling Cytokeratin 6 with Purified ab52635 at 1/100 dilution. Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 μg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1/1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.
All lanes: Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635) at 1/10000 dilution (purified)

Lane 1: Human fetal skin lysates
Lane 2: Rat skin lysates
Lane 3: Mouse skin lysates

Lysates/proteins at 20 µg per lane.

Secondary
All lanes: Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 62 kDa
Observed band size: 62 kDa

 Blocking and diluting buffer: 5% NFDM/TBST.
The lysates were prepared in 1% SDS Hot lysis method.

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma) cells labelling Cytokeratin 5 with purified ab52635 at 1/20 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor®488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
Different batches of ab52635 were tested on Rat skin lysate at 1.0 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 62 kDa.

**Colocalization of KRT5, KRT6 and KRT17 in HSC3 cells**

Immunocytochemistry in HSC3 (human oral squamous carcinoma cell line) cells. Scale bar, 10 μm.

(Taken from Figure S3 of Khanom et al)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat skin tissue sections labeling Cytokeratin 5 with Purified ab52635 at 1:200 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse skin tissue sections labeling Cytokeratin 5 with Purified ab52635 at 1:200 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
Unpurified ab52635 showing positive staining in squamous cell cervical carcinoma tissue.

A431 cells stained with unpurified ab52635 at 1/100 - 1/250

Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635) at 1/10000 dilution (unpurified) + A431 cell lysate at 10 µg

Secondary
Goat anti-rabbit HRP at 1/2000 dilution

Predicted band size: 62 kDa
Observed band size: 62 kDa
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

Human transitional urinary bladder carcinoma stained with unpurified ab52635 at 1/100 - 1/250 dilution.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

Unpurified ab52635 showing positive staining in basal cell breast carcinoma tissue.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

Unpurified ab52635 showing negative staining in ductal breast carcinoma tissue.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 5 antibody [EP1601Y] - Cytoskeleton Marker (ab52635)

Unpurified ab52635 showing negative staining in stomach adenocarcinoma tissue.

Unpurified ab52635 showing positive staining in normal tonsil squamous cells tissue.

Unpurified ab52635 showing positive staining in squamous cell lung carcinoma tissue.
Anti-Cytokeratin 5 antibody [EP1601Y] -
Cytoskeleton Marker (ab52635)

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## Overview

**Product name**  
Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker

**Description**  
Rabbit monoclonal [EP1628Y] to Cytokeratin 8 - Cytoskeleton Marker

**Host species**  
Rabbit

**Tested applications**  
Suitable for: Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF

**Species reactivity**  
Reacts with: Mouse, Rat, Human

**Immunogen**  
Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

**Positive control**  
IHC-P: Human breast adenocarcinoma, ovarian carcinoma, breast carcinoma, colon adenocarcinoma, endometrial carcinoma and thyroid carcinoma tissue; mouse liver tissue; ICC/IF: HT-29 and HeLa cells; WB: HeLa, A431 and HaCaT cell lysates; Human breast cancer lysates and Mouse colon lysate; Flow Cyt (intra): HeLa cells.

**General notes**  
This product is a recombinant monoclonal antibody, which offers several advantages including:
- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this species. Please contact us for more information.

### Properties

**Form**  
Liquid
Storage instructions
Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

Dissociation constant ($K_D$)
$K_D = 4.60 \times 10^{-10} \text{ M}$

Storage buffer
pH: 7.20
Preservative: 0.01% Sodium azide
 Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity
Protein A purified

Clonality
Monoclonal

Clone number
EP1628Y

Isotype
IgG

Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab53280 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application     | Abreviars | Notes |
|-----------------|-----------|-------|
| Flow Cyt (Intra)| ab172730  | $1/20$. Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |
| WB              |           | $1/10000$. Detects a band of approximately 52 kDa (predicted molecular weight: 54 kDa). For unpurified use at 1/25,000 - 1/50,000. |
| IP              |           | $1/20$. For unpurified use at 1:70. |
| IHC-P           |           | $1/250$. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| ICC/IF          |           | $1/100 - 1/500$. |

Target

Function
Together with KRT19, helps to link the contractile apparatus to dystrophin at the costameres of striated muscle.

Tissue specificity
Observed in muscle fibers accumulating in the costameres of myoplasm at the sarcolemma membrane in structures that contain dystrophin and spectrin. Expressed in gingival mucosa and hard palate of the oral cavity.

Involvement in disease
Cirrhosis
Sequence similarities

Belongs to the intermediate filament family.

Post-translational modifications

Phosphorylation on serine residues is enhanced during EGF stimulation and mitosis. Ser-74 phosphorylation plays an important role in keratin filament reorganization.

O-glycosylated. O-GlcNAcylation at multiple sites increases solubility, and decreases stability by inducing proteasomal degradation.

O-glycosylated (O-GlcNAcylated), in a cell cycle-dependent manner.

Cellular localization

Cytoplasm. Nucleus, nucleoplasm. Nucleus matrix.

Images

**All lanes**: Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/10000 dilution

**Lane 1**: A431 cell lysate

**Lane 2**: MCF7 cell lysate

**Lane 3**: Wild-type HeLa cell lysate

**Lane 4**: KRT8 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

**Predicted band size**: 54 kDa

**Lanes 1 - 4**: Merged signal (red and green). Green - ab53280 observed at 55 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab53280 was shown to react with Cytokeratin 8 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255400 (knockout cell lysate ab263785) was used. Wild-type and Cytokeratin 8 knockout samples were subjected to SDS-PAGE. ab53280 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 10000 (For unpurified use at 1/25,000 - 1/50,000) dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.
Immunocytochemistry/ Immunofluorescence analysis of HT-29 (Human colorectal adenocarcinoma epithelial cell) cells labeling Cytokeratin 9 with Purified ab53280 at 1:500 dilution. Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 μg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Alexa Fluor® 488 (ab192467) and Alexa Fluor® 647 (ab192468) conjugated versions are available for this clone.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human thyroid carcinoma tissue sections labeling Cytokeratin 8 with Purified ab53280 at 1:250 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling Cytokeratin 8 with purified ab53280 at 1/20 dilution (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue). Alexa Fluor® 488 (ab192467) and Alexa Fluor® 647 (ab192468) conjugated versions are available for this clone.

**All lanes**: Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/10000 dilution (purified)

**Lane 1**: A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates

**Lane 2**: Human breast cancer lysates

**Lane 3**: HaCaT (Human skin keratinocyte) whole cell lysates

**Lane 4**: Mouse colon lysates

Lysates/proteins at 20 µg per lane.

**Secondary**

**All lanes**: Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

**Predicted band size**: 54 kDa

Blocking and diluting buffer: 5% NFDM/TBST.
ab53280 (purified) at 1:20 dilution (0.2μg) immunoprecipitating Cytokeratin 8 in HeLa whole cell lysate.

**Lane 1 (input):** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate, 10μg

**Lane 2 (+):** ab53280 & HeLa whole cell lysate

**Lane 3 (-):** Rabbit monoclonal IgG (ab172730) instead of ab53280 in HeLa whole cell lysate

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1:1000 dilution.
Blocking and diluting buffer: 5% NFDM/TBST.

**Panx1−/− mice have normal mammary gland epithelial differentiation at lactation**

Immunofluorescent analysis of luminal epithelial marker keratin 8 (green) and myoepithelial marker keratin14 (red) revealed a similar staining pattern in Panx1−/− mice compared to control mice during lactation. Paraaffin-embedded tissue samples.

Hoescht (blue) denotes nuclei. N = 6. Scale bars = 50 um.
Immunocytochemistry/Immunofluorescence analysis of HeLa (human cervix adenocarcinoma) labelling Cytokeratin 8 with purified ab53280 at 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilised by 0.1% tritonX-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Unpurified ab53280 (1:250) staining human Cytokeratin 8 in human breast adenocarcinoma tissue by immunohistochemistry using paraffin embedded tissue.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Cytokeratin 8 with Purified ab53280 at 1:250 dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). Tissue was counterstained with Hematoxylin. ImmunoHistoProbe one step HRP Polymer (ready to use) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control.

Anti-Cytokeratin 8 antibody [EP1628Y] - Cytoskeleton Marker (ab53280) at 1/50000 dilution (unpurified) + A431 cell lysate at 10 µg

**Secondary**
Goat anti-Rabbit HRP labeled at 1/2000 dilution

**Predicted band size:** 54 kDa  
**Observed band size:** 52 kDa
Unpurified ab53280 staining Cytokeratin 8 in Mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formalin and blocked with 10% serum for 20 minutes at 23°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/75 in TBS + 1% BSA) for 1 hour at 23°C. A Biotin-conjugated Goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

Immunofluorescent staining of HeLa cells using unpurified ab53280 (1:100).
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Cytokeratin 8 antibody (EP1628Y) - Cytoskeleton Marker (ab53280)
This image is courtesy of Dr. Shaohua Li

Image: Courtesy of Dr. Shaohua Li, UMDNJ-Robert Wood Johnson Medical School
Sample: mouse embryonic stem cell-differentiated embryoid bodies (EBs)
Preparation:
Fix in 3%PFA in PBS for 30 min at RT
Incubate in 7.5% sucrose-PBS for 3h at RT
Incubate in 15% sucrose-PBS at 4 degree Celsius overnight
Embed the EBs in tissue-Tek OCT compound
Cut frozen sections to 4-20 µm thickness
Primary antibody 1: Rabbit anti cytokeratin 8 (unpurified ab53280), 1:100
Primary antibody 2: Rat anti-perlecan, 1:100
Secondary antibody 1: Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488) pre-adsorbed (ab150081), 1:200
Secondary antibody 2: Goat polyclonal Secondary Antibody to Rat IgG - H&L (Cy5®) pre-adsorbed (ab150081), 1:200
Nuclei were counterstained with DAPI

Fluorescent immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue using unpurified ab53280. Green-CK8 red-PI.
Fluorescent immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using unpurified ab53280. Green-CK8 red-PI.

Fluorescent immunohistochemical analysis of paraffin-embedded human colonic adenocarcinoma tissue using unpurified ab53280. Green-CK8 red-PI.

Fluorescent immunohistochemical analysis of paraffin-embedded human endometrial carcinoma tissue using unpurified ab53280. Green-CK8 red-PI.
Overlay histogram showing HeLa cells stained with unpurified ab53280 (red line). The cells were fixed with 2% PFA (room temperature, 30 min) and then permeabilized with 1% FACS permeabilizing solution for 30 min. The cells were then incubated in 3% FBS in 1X PBS followed by the antibody (ab53280, 1/20 dilution) for 1 hour at room temperature. The cells were then incubated for 30 min at room temperature with the secondary antibody. An isotype control antibody (black line) was used and an unlabelled sample (blue line) was also used as a control.

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D
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P16-INK4A Polyclonal antibody

Catalog Number: 10883-1-AP

Basic Information

- **Catalog Number:** 10883-1-AP
- **Size:** 150μl, Concentration: 500 μg/ml by Nanodrop and 367 μg/ml by Bradford method using BSA as the standard
- **Source:** Rabbit
- **Isotype:** IgG
- **Immunogen Catalog Number:** AG1328
- **GenBank Accession Number:** BC021998
- **GeneID (NCBI):** 1029
- **Full Name:** cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)
- **Calculated MW:** 16 kDa
- **Observed MW:** 16-18 kDa
- **Purification Method:** Antigen affinity purification
- **Recommended Dilutions:**
  - WB: 1:1000-1:8000
  - IP: 0.5-4.0 μg for IP and 1:500-1:2000 for WB
  - IHC: 1:1000-1:4000

Applications

- **Tested Applications:** FC, IHC, IP, WB, ELISA
- **Cited Applications:** CoIP, IP, WB
- **Species Specificity:** human
- **Cited Species:** human, monkey

- **Note-IHC:** suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0

CDKN2A generates several transcript variants which differ in their first exons. At least three alternatively-spliced variants encoding distinct proteins were reported. Two of them named p16-INK4 and p14 are sharing 50% identity. The third one -p14(ARF) is entirely unrelated. 10883-1-AP reacts with p16 specifically. P16 plays an essential role in regulating the cell cycle, and mutations in p16 increase the risk of developing various cancers, including melanoma.

Notable Publications

| Author       | Pubmed ID | Journal                   | Application |
|--------------|-----------|---------------------------|-------------|
| Shin Hamada  | 28971839  | Am J Physiol Gastrointest Liver Physiol | IHC         |
| Julie Wang   | 26416809  | Circulation               | WB          |
| Shengya Tian | 31562192  | Life Sci Alliance         | WB          |

Storage

- **Storage:** Store at -20°C. Stable for one year after shipment.
- **Storage Buffer:** PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
- **Aliquoting is unnecessary for -20°C storage**

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Selected Validation Data

Various lysates were subjected to SDS PAGE followed by western blot with 10883-1-AP (P16-INK4A antibody) at dilution of 1:4000 incubated at room temperature for 1.5 hours.

WB result of p16-INK4A antibody (10883-1-AP, 1:2,000) with si-Control and si-p16 transfected HEK-293 cells.

Immunohistochemical analysis of paraffin-embedded human cervical cancer tissue slide using 10883-1-AP (P16-INK4A antibody) at dilution of 1:2000 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).

IP result of anti-P16-INK4A (IP:10883-1-AP, 4ug; Detection:10883-1-AP 1:1000) with HEK-293 cells lysate 1200 ug.

Immunohistochemical analysis of paraffin-embedded human cervical cancer tissue slide using 10883-1-AP (P16-INK4A antibody) at dilution of 1:2000 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).

1X10^6 HeLa cells were stained with .2ug P16-INK4A antibody (10883-1-AP, red) and control antibody (blue). Fixed with 90% MeOH blocked with 3% BSA (30 min). Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) with dilution 1:1000.
Basic Information

Catalog Number: 10355-1-AP

Size: 150μl, Concentration: 900μg/ml by Nanodrop and 387μg/ml by Bradford method using BSA as the standard

Source: Rabbit

Isotype: IgG

Immunogen Catalog Number: AG0568

GenBank Accession Number: BC000275

GeneID (NCBI): 1026

Full Name: cyclin-dependent kinase inhibitor 1A (p21, Cip1)

Calculated MW: 21 kDa

Observed MW: 21 kDa

Purification Method: Antigen affinity purification

Recommended Dilutions:
WB: 1:1000-1:4000
IP: 0.5-4.0 μg for IP and 1:500-1:2000 for WB
IHC: 1:200-1:600
IF: 1:200-1:600

Applications

Tested Applications: IF, IHC, IP, WB, ELISA

Cited Applications: CoIP, FC, IF, IHC, IP, WB

Species Specificity: human

Cited Species: Artemia sinica, canine, human, pig

Note-IHC: suggested antigen retrieval with TE buffer pH 9.0; (*) Alternatively, antigen retrieval may be performed with citrate buffer pH 6.0

Background Information

CDKN1A (p21, CIP1, WAF1) is a cyclin-dependent kinase inhibitor. CDKN1A binds to and inhibits the activity of cyclin-CDK2 or -CDK4 complexes, and thus functions as a regulator of cell cycle progression at the G1 phase. The expression of CDKN1A is induced by wild-type but not mutant p53 protein, through which CDKN1A mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli. CDKN1A can interact with proliferating cell nuclear antigen (PCNA), and plays a regulatory role in S phase DNA replication and DNA damage repair. CDKN1A was reported to be specifically cleaved by CASP3-like caspases, which thus leads to a dramatic activation of CDK2, and may be instrumental in the execution of apoptosis following caspase activation. Two alternatively spliced variants, which encode an identical protein, have been reported.

Notable Publications

| Author   | Pubmed ID | Journal          | Application |
|----------|-----------|------------------|-------------|
| Jing Zhao | 32986180  | Biotechnol Lett  | WB          |
| Shengya Tian | 31562192 | Life Sci Alliance | WB         |
| Wei Zhao  | 29212181  | Oncotarget       | WB          |

Storage

Storage: Store at -20°C. Stable for one year after shipment.

Storage Buffer: PBS with 0.02% sodium azide and 50% glycerol pH 7.3.

Aliquoting is unnecessary for -20°C storage

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Various lysates were subjected to SDS PAGE followed by western blot with 10355-1-AP (P21 antibody) at dilution of 1:2000 incubated at room temperature for 1.5 hours.

Immunofluorescent analysis of (4% PFA) fixed MCF-7 cells using 10355-1-AP (P21;CDKN1A antibody) at dilution of 1:50 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG( H+L).

IF result of anti-p21 (CDKN1A, 10355-1-AP) in the myotome of a somite at E11.5 by Dr. Zalc A. and Dr. Relaix F. (Red = p21; Green=Pax3 from a GFP reporter; Blue=DAPI).

Immunohistochemical analysis of paraffin-embedded human cervical cancer tissue slide using 10355-1-AP (P21;CDKN1A Antibody) at dilution of 1:400 (under 10x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).

Immunohistochemical analysis of paraffin-embedded human cervical cancer tissue slide using 10355-1-AP (P21;CDKN1A Antibody) at dilution of 1:400 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).

IP result of anti-P21;CDKN1A (IP:10355-1-AP, 4ug; Detection:10355-1-AP 1:1000) with MCF-7 cells lysate 2800 ug.
**Basic Information**

| Catalog Number: 10442-1-AP | GenBank Accession Number: BC003596 |
|-----------------------------|-----------------------------------|
| Source: Rabbit              | GenelD (NCBI): 7157               |
| Isotype: IgG                | Full Name: tumor protein p53      |
| Immunogen Catalog Number:  AG0698 | Calculated MW: 44 kDa          |
|                             | Observed MW: 53 kDa               |

**Purification Method:** Antigen affinity purification

**Recommended Dilutions:**
- WB: 1:5000-1:50000
- IP: 0.5-4.0 ug for IP and 1:500-1:2000 for WB
- IHC: 1:50-1:500
- IF: 1:50-1:500

**Applications**

**Tested Applications:** IF, IHC, IP, WB, ELISA

**Cited Applications:** ChIP, CoIP, IF, IHC, IP, WB

**Species Specificity:** human, rat, mouse

**Cited Species:** Artemia sinica, chicken, goat, human, pig, rat, sheep

**Positives Controls:**
- WB: A431 cells, SMMC-7721 cells, MCF-7 cells, rat colon tissue, mouse spleen tissue, HEK-293 cells, mouse colon tissue, HT-29 cells
- IP: A431 cells
- IHC: human endometrial cancer tissue,
- IF: HepG2 cells, A431 cells

**Background Information**

TP53, also known as P53 and NY-CO-13, belongs to the p53 family and has 9 isoforms. In SDS-Page, the observed molecular weight is about 53 kDa. TP53 acts as a tumor suppressor in many tumor types, including growth arrest or apoptosis depending on the physiological circumstances and cell types. It is involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process. TP53 localizes in the nucleus in most cells but found in the cytoplasm in some cells. (PMID: 26166714; PMID: 25225161)

**Notable Publications**

| Author         | Pubmed ID | Journal       | Application |
|----------------|-----------|---------------|-------------|
| Xiaoyan Liu    | 31574968  | Int J Mol Sci | WB          |
| Shanshan Hu    | 33134183  | Front Oncol   | WB          |
| Fabao Liu      | 26309161  | Oncotarget    | WB          |

**Storage**

Store at -20°C. Stable for one year after shipment.

**Storage Buffer:** PBS with 0.02% sodium azide and 50% glycerol pH 7.3.

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Various lysates were subjected to SDS PAGE followed by western blot with 10442-1-AP (P53 antibody) at dilution of 1:3000 incubated at room temperature for 1.5 hours.

WB result of P53 antibody (10442-1-AP; 1:5000; incubated at room temperature for 1.5 hours) with sh-Control and sh-P53 transfected A431 cells.

Immunofluorescent analysis of (4% PFA) fixed HepG2 cells using 10442-1-AP (P53 antibody) at dilution of 1:50 and Alexa Fluor 488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).

Various lysates were subjected to SDS PAGE followed by western blot with 10442-1-AP (P53 antibody) at dilution of 1:20000 incubated at room temperature for 1.5 hours.

IP Result of anti-P53 (IP:10442-1-AP, 4ug; Detection:10442-1-AP 1:1000) with A431 cells lysate 3000ug.

Immunofluorescent analysis of (4% PFA) fixed A431 cells using P53 antibody (10442-1-AP) at dilution of 1:400 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), CL594-Phalloidin (red).

Immunofluorescent analysis of (4% PFA) fixed A431 cells using P53 antibody (10442-1-AP) at dilution of 1:600 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), CL594-Phalloidin (red).

Immunofluorescent analysis of (4% PFA) fixed A431 cells using P53 antibody (10442-1-AP) at dilution of 1:600 and CoraLite®488-Conjugated AffiniPure Goat Anti-Rabbit IgG(H+L), CL594-Phalloidin (red).

Immunohistochemical analysis of paraffin-embedded human endometrial cancer tissue slide using 10442-1-AP (P53 antibody) at dilution of 1:200 (under 40x lens). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0).
NF-κB p65 Polyclonal antibody

Catalog Number: 10745-1-AP

**Basic Information**

- **Catalog Number**: 10745-1-AP
- **Size**: 150 μl, Concentration: 520 μg/ml by Nanodrop
- **Source**: Rabbit
- **Isotype**: IgG
- **Immunogen Catalog Number**: AG1199
- **GenBank Accession Number**: BC011603
- **GeneID (NCBI)**: 5970
- **Full Name**: v-rel reticuloendotheliosis viral oncogene homolog A (avian)
- **Calculated MW**: 65 kDa
- **Observed MW**: 65 kDa
- **Purification Method**: Antigen affinity purification
- **Recommended Dilutions**:
  - **WB**: 1:500-1:2000
  - **IP**: 0.5-4.0 μg for IP and 1:500-1:2000 for WB
  - **IHC**: 1:50-1:500
  - **IF**: 1:50-1:500

**Applications**

- **Tested Applications**: FC, IF, IHC, IP, WB, ELISA
- **Cited Applications**: chIP, CoIP, IF, IHC, IP, WB
- **Species Specificity**: human
- **Cited Species**: Bombyx mori, bovine, canine, chicken, fish, hamster, human, monkey, pig

*Note* - IHC: suggested antigen retrieval with **TE buffer pH 9.0**; (*) Alternatively, antigen retrieval may be performed with **citrate buffer pH 6.0**

**Positive Controls**:
- **WB**: A431 cells, Jurkat cells, MCF-7 cells, K-562 cells, HeLa cells
- **IP**: HeLa cells
- **IHC**: human breast cancer tissue, human stomach tissue, human liver cancer tissue
- **IF**: HepG2 cells

**Background Information**

Nuclear factor κ B (NF-κB) is a sequence-specific DNA-binding protein complex which regulates the expression of viral genomes, including the human immunodeficiency virus, and a variety of cellular genes, particularly those involved in immune and inflammatory responses. The members of the NF-κB family in mammalian cells include the proto-oncogene c-Rel, p50/p105 (NFκB1), p65 (RelA), p52/p100 (NFκB2), and RelB. All of these proteins share a conserved 300-amino acid region known as the Rel homology domain which is responsible for DNA binding, dimerization, and nuclear translocation of NF-κB. The p65 subunit is a major component of NF-κB complexes and is responsible for trans-activation. NF-κB heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-κB p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of IκB upon NF-κB the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-κB complex. It associates with chromatin at the NF-κB promoter region via association with DDX1. This antibody is a rabbit polyclonal antibody raised against residues near the N terminus of human RELA.

**Notable Publications**

| Author         | Pubmed ID | Journal        | Application |
|----------------|-----------|----------------|-------------|
| Yanliang Wu    | 34601083  | J Ethnopharmacol | WB          |
| Kun-Yi Li      | 32920833  | J Neurochem     | WB          |
| Ziliu Zhang    | 34570444  | Cancer Biol Med | IF, WB      |

**Storage**

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- **Storage Buffer**: PBS with 0.02% sodium azide and 50% glycerol pH 7.3.
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Selected Validation Data

WB result of RELA, p65 antibody (10745-1-AP, 1:500) with si-Control and si-RELA, p65 transfected HeLa cells.

Various lysates were subjected to SDS PAGE followed by western blot with 10745-1-AP (NF-κB p65 antibody) at dilution of 1:1000 incubated at room temperature for 1.5 hours.

IP Result of anti-p65 (IP:10745-1-AP, 3ug; Detection:10745-1-AP 1:1000) with HeLa cells lysate 5000ug.

Immunohistochemical analysis of paraffin-embedded human breast cancer using 10745-1-AP (p65 antibody) at dilution of 1:100 (under 40x lens).

Immunofluorescent analysis of (-20°C Ethanol) fixed HepG2 cells using 10745-1-AP (p65; RELA antibody) at dilution of 1:100 and Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L).

1X10^6 HeLa cells were stained with 0.2ug p65; RELA antibody (10745-1-AP, red) and control antibody (blue). Fixed with 90% MeOH blocked with 3% BSA (30 min). Alexa Fluor 488-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) with dilution 1:1000.
Human Periostin/OSF-2 Antibody
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3548

DESCRIPTION
Species Reactivity: Human
Specificity: Detects human Periostin/OSF-2 in direct ELISAs and Western blots. In direct ELISAs, less than 40% cross-reactivity with recombinant mouse Periostin and recombinant rat Periostin is observed.
Source: Polyclonal Goat IgG
Purification: Antigen Affinity-purified
Immunogen: Mouse myeloma cell line NS0-derived recombinant human Periostin Asn22-Gln836; Accession # Q15063
Endotoxin Level: <0.10 EU per 1 μg of the antibody by the LAL method.
Formulation: Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.
*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

| Recommended Concentration | Sample |
|---------------------------|--------|
| Western Blot              | 1 µg/mL See Below |
| Immunohistochemistry      | 5-15 µg/mL See Below |
| Immunoprecipitation       | 25 µg/mL See Below |

DATA
Western Blot
Detection of Human Periostin/OSF-2 by Western Blot. Western blot shows lysates of human breast cancer tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human Periostin/OSF-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3548) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for Periostin/OSF-2 at approximately 90-95 kDa (as indicated). This experiment was conducted using Immunoblot Buffer Group 8.

Immunoprecipitation
Immunoprecipitation of Human Periostin/OSF-2. Human Periostin/OSF-2 was immunoprecipitated from human milk samples diluted in 1X Sample Diluent Concentrate 2 (Catalog # DYC002) and incubated with 3 µg Goat Anti-Human Periostin/OSF-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3548) or Normal Goat IgG Control (Catalog # AB-108-C) plus 30 µL Protein G beads overnight. Immunoprecipitated Periostin/OSF-2 was detected by Western blot under reducing conditions using 1 µg/mL Goat Anti-Human Periostin/OSF-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3548). View our recommended buffer recipes for immunoprecipitation.

Immunohistochemistry
Periostin/OSF-2 in Human Breast.
Periostin/OSF-2 was detected in immersion fixed paraffin-embedded sections of human breast using Goat Anti-Human Periostin/OSF-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3548) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to stromal cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.
**Human Periostin/OSF-2 Antibody**

Antigen Affinity-purified Polyclonal Goat IgG  
Catalog Number: AF3548

**PREPARATION AND STORAGE**

| Reconstitution | Reconstitute at 0.2 mg/mL in sterile PBS. |
|----------------|-----------------------------------------|
| Shipping       | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C.* |
| Stability & Storage | Use a manual defrost freezer and avoid repeated freeze-thaw cycles. |
|                 | • 12 months from date of receipt, -20 to -70 °C as supplied. |
|                 | • 1 month, 2 to 8 °C under sterile conditions after reconstitution. |
|                 | • 6 months, -20 to -70 °C under sterile conditions after reconstitution. |

**BACKGROUND**

Human OSF-2 (Osteoblast-Specific Factor 2), also known as Periostin, is a 170 kDa secreted homodimeric protein that belongs to the periostin family of the FAS1 superfamily of molecules. It is a TGF-β inducible molecule that serves as both an adhesion molecule and tumor suppressor. It is synthesized as an 836 amino acid (aa) precursor that contains a 21 aa signal sequence and an 815 aa mature region. It is unknown if the molecule has any significant glycosylation. The human homodimer is not disulfide-linked. The molecule consists of two distinct regions. The N-terminus contains an 55 aa EMI domain, while the C-terminus contains four 130 aa Fasciculin type 1 (FAS1) domains. The EMI domain is cysteine-rich and shows a highly basic α-helix. Each FAS1 repeat exhibits a novel seven-stranded β-wedge with a multiple α-helical fold. Three alternate splice forms are known that are C-terminal to the fourfold FAS1 repeats. These mature molecules are 758 and 761 aa in length. The first shows a one aa substitution for aa 649-706 of the mature molecule. The second shows a one aa substitution for aa 649-676, and a deletion of 27 aa between aa 784-810 of the mature molecule. The significance of the alternate splice forms is not clear. OSF-2 is known to bind to α\(v\)β3 and α\(v\)β5 integrins. It is synthesized by smooth muscle cells, fibroblasts, osteoblasts, and multiple carcinoma cell types. OSF-2 induces expression of VEGFR2/KDR on endothelial cells (EC) by binding to EC α\(v\)β5. It also promotes cell transformation to a tumorigenic phenotype, accompanied by MMP-9 and fibronectin production and cell migration. Mature human OSF-2 is 91%, 96% and 91% aa identical to rat, dog, and mouse OSF-2, respectively.
Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb

For Research Use Only. Not For Use In Diagnostic Procedures.

| Applications | Reactivity | Sensitivity | MW (kDa) | Source/Isotype | UniProt ID | Entrez-Gene Id |
|--------------|------------|-------------|----------|----------------|------------|----------------|
| WB, IF-IC, FC-FP | H, M, R | Endogenous | 60 | Rabbit | Q99717, Q15797 | 4090, 4086 |

**Product Usage Information**

**Application**
- Western Blotting: 1:1000
- Immunofluorescence (Immunocytochemistry): 1:800
- Flow Cytometry (Fixed/Permeabilized): 1:800

**Storage**
Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at −20°C. Do not aliquot the antibody.

**Specificity / Sensitivity**
Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb detects endogenous levels of Smad1 and Smad5 only when dually phosphorylated at Ser463 and Ser465 and is also predicted to detect Smad9 (Smad8) when phosphorylated at Ser465 and Ser467. The antibody does not cross-react with other Smad-related proteins.

**Species Reactivity**
Human, Mouse, Rat

**Source / Purification**
Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser463/465 of human Smad5.

**Background**
Bone morphogenetic proteins (BMPs) constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell fate determination, proliferation, differentiation, and apoptosis (1,2). BMP receptors are members of the TGF-β family of Ser/Thr kinase receptors. Ligand binding induces multimerization, autophosphorylation, and activation of these receptors (3-5). They subsequently phosphorylate Smad1 at Ser463 and Ser465 in the carboxy-terminal motif SSXS, as well as Smad5 and Smad9 (Smad8) at their corresponding sites. These phosphorylated Smads dimerize with the coactivating Smad4 and translocate to the nucleus, where they stimulate transcription of target genes (5).

MAP kinases and CDKs 8 and 9 phosphorylate residues in the linker region of Smad1, including Ser206. The phosphorylation of Ser206 recruits Smurf1 to the linker region and leads to the degradation of Smad1 (6). Phosphorylation of this site also promotes Smad1 transcriptional action by recruiting YAP to the linker region (7).

1. Hogan, B.L. (1996) Genes Dev 10, 1580-94.
2. Hoodless, P.A. et al. (1996) Cell 85, 489-500.
3. Klemm, J.D. et al. (1998) Annu Rev Immunol 16, 569-92.
4. Kretzschmar, M. et al. (1997) Genes Dev 11, 984-95.
5. Whitman, M. (1998) Genes Dev 12, 2445-62.
6. Sapkota, G. et al. (2007) Mol Cell 25, 441-54.
7. Alarcón, C. et al. (2009) Cell 139, 757-69.
#9516

Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb

Western blot analysis of extracts from untreated or BMP-4-treated HeLa or NIH/3T3 cells using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb.

Flow cytometric analysis of HeLa cells, untreated (blue) or BMP-treated (green), using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb compared to a nonspecific negative control antibody (red).

Confocal immunofluorescent analysis of HT1080 cells, serum-starved (left) or serum-starved then treated with hBMP2 (50 ng/ml, 30 min; right) using Phospho-Smad1/5 (Ser463/465) (41D10) Rabbit mAb (green) and Cox IV (4D11-B3-E8) Mouse mAb #11967 (red).

Confocal immunofluorescent analysis of HT-1080 cells, BMP-treated (left) and untreated (right), using Phospho-Smad5 (Ser463/Ser465) (41D10) Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).
Phospho-Smad1/5 (Ser463/465) (41D10)
Rabbit mAb

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Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb

For Research Use Only. Not For Use In Diagnostic Procedures.

**Product Usage Information**

| Applications | Reactivity | Sensitivity | MW (kDa) | Source/Igotype | UniProt ID | Entrez-Gene Id |
|--------------|------------|-------------|----------|----------------|------------|---------------|
| WB           | H M R Mk   | Endogenous  | 52, 80   | Rabbit IgG     | P84022, Q15796 | 4088, 4087   |

**Storage**

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

**Specificity / Sensitivity**

Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb recognizes endogenous levels of Smad2 protein when phosphorylated at Ser465 and Ser467. This antibody also recognizes endogenous levels of Smad3 protein when phosphorylated Ser422 only or at both Ser423 and Ser425.

**Species Reactivity:**

Human, Mouse, Rat, Monkey

**Source / Purification**

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser465/467 of human Smad2 protein.

**Background**

Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmit TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 2, 3, 5, and 8; the common-mediator Smad (co-Smad), Smad4; and the antagonistic or inhibitory Smads (I-Smads), Smad6 and 7 (1-5). Activated type I receptors associate with specific R-Smads and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-Smad dissociates from the receptor and forms a heteromeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, Smads can target a variety of DNA binding proteins to regulate transcriptional responses (6-8).

1. Heldin, C.H. et al. (1997) Nature 390, 465-71.
2. Attisano, L. and Wrana, J.L. (1998) Curr Opin Cell Biol 10, 188-94.
3. Derynck, R. et al. (1998) Cell 95, 737-40.
4. Massagué, J. (1998) Annu Rev Biochem 67, 753-91.
5. Whitman, M. (1998) Genes Dev 12, 2445-62.
6. Wu, G. et al. (2000) Science 287, 92-7.
7. Attisano, L. and Wrana, J.L. (2002) Science 296, 1646-7.
8. Moustakas, A. et al. (2001) J Cell Sci 114, 4359-69.

**Applications:**

- **WB:** Western Blotting

**Dilution:**

1:1000

**IMPORTANT:** For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

**CROSS-REACTIVITY KEY**

- H: human
- M: mouse
- R: rat
- Hm: hamster
- Mk: monkey
- Vr: virus
- Mi: mink
- C: chicken
- Dm: D. melanogaster
- X: Xenopus
- Z: zebrafish
- B: bovine
- Dg: dog
- Pg: pig
- Sc: S. cerevisiae
- Ce: C. elegans
- Hr: horse
- Rab: rabbit
- All: all species expected

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#8828

**Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb**

Western blot analysis of extracts from HaCaT cells, untreated (-) or treated with hTGF-β3 #8425 (+) in the absence or presence of the TGFR inhibitor SB 431542, using Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb (upper) or Smad2/3 (D7G7) XP® Rabbit mAb #8685 (lower).
#8828

Phospho-Smad2 (Ser465/467)/Smad3 (Ser423/425) (D27F4) Rabbit mAb

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Smad1 (D59D7) XP® Rabbit mAb

For Research Use Only. Not For Use In Diagnostic Procedures.

Applications: WB, IP, ChIP
Reactivity: H M Mk
Sensitivity: Endogenous
MW (kDa): 60
Source/Isotype: Rabbit IgG
UniProt ID: P15797
Entrez-Gene Id: 4086

Product Usage Information

For optimal ChIP results, use 5 μl of antibody and 10 μg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP® Enzymatic Chromatin IP Kits.

Application Dilution
Western Blotting 1:1000
Immunoprecipitation 1:100
Chromatin IP 1:100

Storage
Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

Specificity / Sensitivity
Smad1 (D59D7) XP® Rabbit mAb recognizes endogenous levels of total Smad1 protein.

Species Reactivity:
Human, Mouse, Monkey

Species predicted to react based on 100% sequence homology:
Xenopus, Bovine

Source / Purification
Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Ser190 of human Smad1 protein.

Background
Bone morphogenetic proteins (BMPs) constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell fate determination, proliferation, differentiation, and apoptosis (1,2). BMP receptors are members of the TGF-β family of Ser/Thr kinase receptors. Ligand binding induces multimerization, autophosphorylation, and activation of these receptors (3-5). They subsequently phosphorylate Smad1 at Ser463 and Ser465 in the carboxy-terminal motif SSXS, as well as Smad5 and Smad9 (Smad8) at their corresponding sites. These phosphorylated Smads dimerize with the coactivating Smad4 and translocate to the nucleus, where they stimulate transcription of target genes (5).

MAP kinases and CDKs 8 and 9 phosphorylate residues in the linker region of Smad1, including Ser206. The phosphorylation of Ser206 recruits Smurf1 to the linker region and leads to the degradation of Smad1 (6). Phosphorylation of this site also promotes Smad1 transcriptional action by recruiting YAP to the linker region (7).

REFERENCES
1. Hogan, B.L. (1996) Genes Dev 10, 1580-94.
2. Hoodless, P.A. et al. (1996) Cell 85, 489-500.
3. Klemm, J.D. et al. (1998) Annu Rev Immunol 16, 569-92.
4. Kretzschmar, M. et al. (1997) Genes Dev 11, 984-95.
5. Whitman, M. (1998) Genes Dev 12, 2445-62.
6. Sapkota, G. et al. (2007) Mol Cell 25, 441-54.
7. Alarcón, C. et al. (2009) Cell 139, 757-69.

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APPLICATIONS KEY
WB: Western Blotting
IP: Immunoprecipitation
ChIP: Chromatin IP

CROSS-REACTIVITY KEY
H: human
M: mouse
R: rat
Hm: hamster
Mk: monkey
Vr: virus
M: mink
C: chicken
Dm: D. melanogaster
X: Xenopus
Z: zebrafish
B: bovine
Dg: dog
Pg: pig
Sc: S. cerevisiae
Ce: C. elegans
Hr: horse
Rab: rabbit
All: all species expected

IMPORTANT: For Western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

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Western blot analysis of extracts from various cell lines using Smad1 (D59D7) XP® Rabbit mAb.

Chromatin immunoprecipitations were performed with cross-linked chromatin from MCF7 cells treated with Human BMP2 #4697 (50 ng/ml) for one hour and either Smad1 (D59D7) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human ID1 Promoter Primers #5139, human SMAD6 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.
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**Smad2/3 (D7G7) XP® Rabbit mAb**

**For Research Use Only. Not For Use In Diagnostic Procedures.**

**Applications:** WB, IP, IF-IC, FC-FP, ChIP, ChIP-seq

**Reactivity:** H M R Mk

**Sensitivity:** Endogenous

**MW (kDa):** 52, 80

**Source/Isotype:** Rabbit IgG

**UniProt ID:** P84022, Q15796

**Entrez-Gene Id:** 4088, 4087

**Product Usage Information**

For optimal ChIP and ChIP-seq results, use 5 μl of antibody and 10 μg of chromatin (approximately 4 x 10^6 cells) per IP. This antibody has been validated using SimpleChIP Enzymatic Chromatin IP Kits.

**Application** | Dilution
--- | ---
Western Blotting | 1:1000
Immunoprecipitation | 1:100
Immunofluorescence (Immunocytochemistry) | 1:400 - 1:800
Flow Cytometry (Fixed/Permeabilized) | 1:100 - 1:400
Chromatin IP | 1:100
Chromatin IP-seq | 1:100

**Storage**

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

**Specificity / Sensitivity**

Smad2/3 (D7G7) XP® Rabbit mAb recognizes endogenous levels of total Smad2/3 protein.

**Species Reactivity:**

Human, Mouse, Rat, Monkey

**Source / Purification**

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding His198 of human Smad2/3 protein.

**Background**

Members of the Smad family of signal transduction molecules are components of a critical intracellular pathway that transmits TGF-β signals from the cell surface into the nucleus. Three distinct classes of Smads have been defined: the receptor-regulated Smads (R-Smads), which include Smad1, 2, 3, 5, and 8; the common-mediator Smad (co-Smad), Smad4; and the antagonistic or inhibitory Smads (I-Smads), Smad6 and 7 (1-5). Activated type I receptors associate with specific R-Smads and phosphorylate them on a conserved carboxy-terminal SSXS motif. The phosphorylated R-Smad dissociates from the receptor and forms a heteromeric complex with the co-Smad (Smad4), allowing translocation of the complex to the nucleus. Once in the nucleus, Smads can target a variety of DNA binding proteins to regulate transcriptional responses (6-8).

1. Heldin, C.H. et al. (1997) Nature 390, 465-71.
2. Attisano, L. and Wrana, J.L. (1998) Curr Opin Cell Biol 10, 188-94.
3. Derynck, R. et al. (1998) Cell 95, 737-40.
4. Massagué, J. (1998) Annu Rev Biochem 67, 753-91.
5. Whitman, M. (1998) Genes Dev 12, 2445-62.
6. Wu, G. et al. (2000) Science 287, 92-7.
7. Attisano, L. and Wrana, J.L. (2002) Science 296, 1646-7.
8. Moustakas, A. et al. (2001) J Cell Sci 114, 4359-69.

**IMPORTANT:** For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
Western blot analysis of extracts from HeLa and ACHN cells using Smad2/3 (D7G7) XP® Rabbit mAb.

Immunoprecipitation of Smad2/3 protein from HCT116 extracts. Lane 1 is 10% input, lane 2 is Rabbit (DA1E) mAb IgG XP® Isotype Control #3900, and lane 3 is Smad2/3 (D7G7) XP®. Western blot analysis was performed using Smad2/3 (D7G7) XP®. Mouse Anti-rabbit IgG (Conformation Specific) (L27A9) mAb #3678 was used as a secondary antibody.

Confocal immunofluorescent analysis of HeLa cells, serum starved (left), treated with hTGF-β3 #8425 (100 ng/ml, 30 min, center), or treated with hTGF-β3 and SB43152 (10 μg/ml, 1 hr, right), using Smad2/3 (D7G7) XP® Rabbit mAb (green). Actin filaments were labeled with DY-554 phalloidin (red).

Flow cytometric analysis of HeLa cells using Smad2/3 (D7G7) XP® Rabbit mAb (solid line) compared to concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed line). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary control.

Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with hTGF-β3 #8425 (7 ng/ml, 1 hr) and Smad2/3 (D7G7) XP® Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared using SimpleChIP® ChiP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across ID1, a known target gene of Smad2/3 (see additional figure containing ChIP-qPCR data). For additional ChiP-seq tracks, please download the product data sheet.

Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with hTGF-β3 #8425 (7 ng/ml, 1 hr) and Smad2/3 (D7G7) XP® Rabbit mAb, using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. DNA Libraries were prepared using SimpleChIP® ChiP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 20 (upper), including ID1 (lower), a known target gene of Smad2/3 (see additional figure containing ChiP-qPCR data).
Chromatin immunoprecipitations were performed with cross-linked chromatin from HaCaT cells treated with hTGF-β3 #8425 (7 ng/ml, 1 hr) and either Smad2/3 (D7G7) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human CDKN1A Intron 1 Primers #4669, SimpleChIP® Human ID1 Promoter Primers #5139, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.
#8685

Smad2/3 (D7G7) XP® Rabbit mAb

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**Smad5 (D4G2) Rabbit mAb**

**Product Usage Information**

For optimal ChIP results, use 5 μl of antibody and 10 μg of chromatin (approximately 4 x 10^5 cells) per IP.

This antibody has been validated using SimpleChIP Enzymatic Chromatin IP Kits.

| Application         | Reactivity | Sensitivity | MW (kDa) | Source/Isoype  | UniProt ID | Entrez-Gene ID |
|---------------------|------------|-------------|----------|----------------|------------|----------------|
| Western Blotting    | 1:1000     |             | 60       | Rabbit IgG     | Q9717      | 4090           |
| Immunoprecipitation | 1:50       |             |          |                |            |                |
| Chromatin IP        | 1:100      |             |          |                |            |                |

**Storage**

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

**Background**

Bone morphogenetic proteins (BMPs) constitute a large family of signaling molecules that regulate a wide range of critical processes including morphogenesis, cell-fate determination, proliferation, differentiation, and apoptosis (1,2). BMP receptors are members of the TGF-β family of Ser/Thr kinase receptors. Ligand binding induces multimerization, autophosphorylation, and activation of these receptors (3-5). They subsequently phosphorylate Smad1 at Ser463 and Ser465 in the carboxy-terminal motif SSXS, as well as Smad5 and Smad9 (Smad8) at their corresponding sites. These phosphorylated Smads dimerize with the coactivating Smad4 and translocate to the nucleus, where they stimulate transcription of target genes (5).

**Specificity / Sensitivity**

Smad5 (D4G2) Rabbit mAb recognizes endogenous levels of total Smad5 protein.

**Species Reactivity:**

Human, Mouse, Rat, Monkey

**Source / Purification**

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Pro249 of human Smad5 protein.

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**APPLICATIONS KEY**

| WB | IP | ChIP |
|----|----|------|
| Western Blotting | Immunoprecipitation | Chromatin IP |

**CROSS-REACTIVITY KEY**

| H | human |
| M | mouse |
| R | rat |
| Hamster | Mic | monkey |
| V | virus |
| Mi | mink |
| C | chicken |
| Dm | D. melanogaster |
| X | Xenopus |
| Z | zebrafish |
| B | bovine |
| Dg | dog |
| P | pig |
| Sc | S. cerevisiae |
| Ce | C. elegans |
| Hr | horse |
| Rab | rabbit |
| All | all species expected |

**IMPORTANT:** For western blots, incubate membrane with diluted primary antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
Western blot analysis of extracts from various cell lines using Smad5 (D4G2) Rabbit mAb.

Immunoprecipitation of Smad5 from HT-1080 cell extracts using Normal Rabbit IgG #2729 (lane 2) or Smad5 (D4G2) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Smad5 (D4G2) Rabbit mAb.

Chromatin immunoprecipitations were performed with cross-linked chromatin from MCF7 cells treated with Human BMP2 #4697 (50 ng/ml, 1 hr) and either Smad5 (D4G2) Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads) #9003. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human ID1 Promoter Primers #5139, human Smad6 promoter primers, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.
#12534

Smad5 (D4G2) Rabbit mAb

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Product Information

Anti-Actin, α-Smooth Muscle antibody, Mouse monoclonal clone 1A4, purified from hybridoma cell culture
Catalog Number A5228

Product Description
Anti-Actin, α-Smooth Muscle antibody, Mouse monoclonal (mouse IgG2a isotype) is derived from the 1A4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with the NH₂ terminal synthetic decapeptide of α-smooth muscle actin, coupled to keyhole limpet hemocyanin (KLH). The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Anti-Actin, α-Smooth Muscle antibody, Mouse monoclonal is specific for the single isoform α-actin using indirect immunofluorescent labeling of formalin-fixed, paraffin-embedded human, rabbit, rat, mouse, bovine, frog, goat, guinea pig, dog, sheep, snake, and chicken tissue sections. This antibody can be used in ELISA, immunobloting, and immunocytochemistry.

The two major cytoskeletal proteins implicated in cell motility are actin and myosin. Actin and myosin are constituents of many cell types and are involved in a myriad of cellular processes including locomotion, secretion, cytoplasmic streaming, phagocytosis, and cytokinesis. Although actin is one of the most conserved eukaryotic proteins, it is expressed in mammals and birds as six isoforms characterized by electrophoresis and amino acid sequence analysis. Four of the six represent differentiation markers of muscle tissues. The other two are found in practically all cells. Actin isoforms show >90% overall sequence homology, but only 50-60% homology in the 18 NH2-terminal residues. The NH2-terminal region of actin appears to be a major antigenic region, and may be involved in the interaction of actin with other proteins such as myosin. It has been shown that the relative proportion of actin isoforms are different in smooth muscles of different organs and change within the same population of smooth muscle cells during development, pathological situations and different culture conditions. The actin in cells of various species and tissue origin are very similar in their immunological and physical properties.

Anti-Actin, α-Smooth Muscle antibody, Mouse monoclonal may help in the characterization of stromal cell heterogeneity in various organs and distinguishing smooth muscle cells from fibroblasts in mixed cultures.

Reagent
Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~2 mg/ml.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots. Repeated freezing and thawing, or storage in frost-free freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile
Indirect immunofluorescence: a working antibody concentration of 5-10 µg/ml is recommended for labeling of blood vessels in formalin-fixed, paraffin-embedded human tonsil or appendix tissue.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilution by titration.

References
1. Skalli, O., et al., J. Cell Biol., 103, 2787-2796 (1986).
2. Abd-El-Basset, E., et al., Neurosci. Lett., 125, 117-120 (1991).
3. Durand-Arczynska, W., et al., Histochemistry, 100, 465-471 (1993).
4. van Royen, N., et al., FASEB, 16, 432-434 (2002).
5. Slaninova, I., et al., *Antonie Van Leeuwenhoek.*, 75, 361-368 (1999).

6. Del Pup, L., et al., *Int. J. Mol. Med.*, 10, 561-568 (2002).
Anti-Versican antibody ab19345

Overview

Product name: Anti-Versican antibody
Description: Rabbit polyclonal to Versican
Host species: Rabbit
Specificity: This antibody can be used to detect versican V1 (ADAMTS-1/4 site). This product is against the neoepitope of versican generated by cleavage of the versican core protein by ATAMTS 1/4.

Tested applications: Suitable for: WB
Species reactivity: Reacts with: Human
Predicted to work with: Rat

Immunogen: Synthetic peptide: CGG- DPEAAE, corresponding to amino acids 436-441 of Human Versican (Peptide available as ab39784.)

General notes:

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form: Liquid
Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
Storage buffer: Preservative: 0.05% Sodium azide
Constituents: PBS, 0.1% BSA
Purity: Protein A purified
Clonality: Polyclonal
Isotype: IgG
The Abpromise guarantee Our Abpromise guarantee covers the use of ab19345 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|-------------|-----------|-------|
| WB          | 🌟🌟🌟🌟🌟 (2) | Use a concentration of 1 µg/ml. Detects a band of approximately 70 kDa. Can be blocked with Human Versican peptide (ab39784). |

Target

Function May play a role in intercellular signaling and in connecting cells with the extracellular matrix. May take part in the regulation of cell motility, growth and differentiation. Binds hyaluronic acid.

Tissue specificity Cerebral white matter and plasma. Isoform V0 and isoform V1 are expressed in normal brain, gliomas, medulloblastomas, schwannomas, neurofibromas, and meningiomas. Isoform V2 is restricted to normal brain and gliomas. Isoform V3 is found in all these tissues except medulloblastomas.

Involvement in disease Defects in VCAN are the cause of Wagner syndrome type 1 (WGN1) [MIM:143200]. WGN is a dominantly inherited vitreoretinopathy characterized by an optically empty vitreous cavity with fibrillary condensations and a preretinal avascular membrane. Other optical features include progressive chorioretinal atrophy, perivascular sheathing, subcapsular cataract and myopia. Systemic manifestations are absent in WGN.

Sequence similarities Belongs to the aggrecan/versican proteoglycan family. Contains 1 C-type lectin domain. Contains 2 EGF-like domains. Contains 1 Ig-like V-type (immunoglobulin-like) domain. Contains 2 Link domains. Contains 1 Sushi (CCP/SCR) domain.

Developmental stage Disappears after the cartilage development.

Post-translational modifications Phosphorylation sites are present in the extracellular medium.

Cellular localization Secreted > extracellular space > extracellular matrix.

Images

Anti-Versican antibody (ab19345) at 1 µg/ml + Lysates prepared from human aorta.

ab19345 has been successfully used in Western blot procedures. By Western blot, this antibody detects an ~70 kDa protein representing Versican from mouse cumulus oocyte complexes.
Please note: All products are “FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES”

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