Supporting Information for

Complete Native Stable Isotope Labeling by Amino Acids of
Saccharomyces cerevisiae for Global Proteomic Analysis

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**Figure S-1.** Effect of exogenous proline on the proteome of BY4741 cells. (a) Addition of exogenous proline to the growth medium does not affect the cells' proteome. BY4741 cells were grown in the presence (+) or absence (-) of external proline (Pro) for quantitative MS analysis using stable isotope peptide dimethyl labeling (n=3). Proteins of the arginine-to-proline conversion (orange) and the arginine biosynthesis (blue) are labeled. The dashed horizontal line indicates a t-test p-value of 0.05. Larger circles indicate proteins significantly down-/up-regulated (i.e. both t-test and Significance B p-value < 0.05). (b) Metabolic conversion of arginine to proline in *S. cerevisiae*. The enzymes Car1, Car2, and Pro3 are not altered in abundance by external proline as shown in (a).
Figure S-2. Effect of exogenous proline on the MS intensities of Pro6-containing peptides in BY4741 cells grown on 2% glucose (a), 2% galactose (b), and 3% glycerol/0.02% glucose (c). Cells were grown in the presence of heavy arginine and lysine with (w/) or without (w/o) addition of exogenous unlabeled proline (Pro0; 200 mg/l). Relative intensities were calculated by dividing the MS intensity of single peptides containing Pro6 by the summed intensity of all peptides identified per dataset; data were log$_{10}$-transformed and the number of peptides per log$_{10}$ intensity bin were calculated. Addition of unlabeled proline to the medium strongly reduces the intensities of identified Pro6-containing peptides.
Figure S-3. Metabolic incorporation of heavy lysine (Lys8) and arginine (Arg10) into proteins of BY4741 wild-type (WT) and deletion strains as indicated. Strains were grown to an OD$_{600}$ of approximately 1.0. Cells were lysed and proteins analyzed by LC-MS. Note that the analyses of mitochondrial gene deletion effects (see Figure 4) were performed in three biological replicates. In one of the replicates, the deletion strain was labeled with heavy lysine and arginine, in the other two replicates, labeling was reversed (i.e. wild-type cells were labeled with heavy amino acids). For the incorporation of heavy lysine and arginine into proteins of wild-type cells, the results of two representative experiments are shown.
Figure S-4. Gene Ontology (GO) term enrichment analysis of proteins significantly altered in abundance in \( \text{sdh5}\Delta \) (a), \( \text{phb1}\Delta \) (b), and \( \text{coi1}\Delta \) (c) cells. P-values after Holm-Bonferroni false discovery rate (FDR < 0.05) correction were plotted against the corresponding GO terms from the domains ‘cellular component’ (cc), ‘molecular function’ (mf), and ‘biological process’ (bp). Numbers highlighted in light grey indicate the number of proteins assigned to the respective term. ↑/↓, proteins with significantly increased/decreased abundance in gene deletion strains compared to wild-type cells.
Table S-5. Extent of heavy arginine-to-proline conversion during native SILAC of cells grown using the indicated carbon sources calculated based on the MS intensity of Pro6-containing peptides. Data rely on the same datasets as data presented in Table 1. Color gradient: low (< 1%; yellow), medium (1 - 10%, shades of orange), high (> 10%; red) arginine-to-proline conversion. Numbers represent the relative intensity of Pro6-containing peptides in % in relation to the summed intensity of all peptides identified per dataset. SD, standard deviation (n=3); OD, optical density at 600 nm; glc, glucose; gal, galactose; gly, glycerol

| C-source | Pro | Rel. intensities of Pro6-containing peptides [% ± SD] | OD 0.5 | OD 1.0 | OD 1.5 | OD 4.0 |
|----------|-----|----------------------------------------------------|--------|--------|--------|--------|
| glc      | -   | 2.9±1.53                                           | 5.3±3.09| 8.1±2.94| 12.9±7.78|
|          | +   | 0.8±0.09                                           | 1.1±0.08| 1.4±0.08| 3.5±0.12|
| gal      | -   | 4.1±0.25                                           | 4.2±0.45| 6.1±0.70| 5.6±1.00|
|          | +   | 0.1±0.07                                           | 1.1±0.36| 2.3±0.29| 5.4±0.23|
| gly/glc | -   | 0.3±0.07                                           | 0.9±0.28| 3.8±1.09| 14.5±1.21|
|          | +   | 0.1±0.07                                           | 0.3±0.09| 0.5±0.07| 3.2±1.24|
Extended Experimental Procedures

Yeast cultivation

Cells were pre-cultured in 30 ml of medium with an initial OD$_{600}$ of 0.1 and grown overnight at 160 rpm and 30°C. Pre-cultured cells were diluted into 150 ml of fresh medium to reach an OD$_{600}$ of 0.025. Cells were further cultivated at 160 rpm and 30°C. Cells were harvested at exponential growth phase (OD$_{600}$ 1.0-1.5) unless stated otherwise.

Preparation of whole cell lysates and mitochondria-enriched fractions

Cells were harvested by centrifugation for 5 min at 5,000 x g and washed with deionized water. For the preparation of whole cell lysates, cells were resuspended in 500 µl of urea buffer (8 M urea, 75 mM NaCl, 50 mM Tris/HCl [pH 8.0], 1 mM EDTA) and lysed by bead-beating (twice for 4 min) using a MiniLys homogenizer (Peqlab) operated at 5,000 rpm. Cell debris was removed by centrifugation for 5 min at 15,000 x g and 4°C.

For the preparation of mitochondria-enriched fractions used for LC-MS analyses, a small-scale protocol was established based on a medium-scale protocol described recently in order to enable simultaneous processing of multiple strains from low culture volumes. Light and heavy SILAC-labeled cells from 100 - 150-ml cultures were mixed in equal ratios based on OD$_{600}$ (100 - 150 OD$_{600}$ units each) and harvested as described above. Cells were washed with dH$_2$O, resuspended in 1.5 ml of DTT buffer (100 mM Tris/H$_2$SO$_4$ [pH 9.4], 10 mM DTT), transferred to 2-ml reaction tubes and incubated for 10 min at 1,000 rpm and 30°C using a thermomixer (Thriller®, Peqlab/Thermo Fisher). After centrifugation for 5 min at 1,500 x g and RT, cells were washed with 1.5 ml of Zymolyase buffer (20 mM potassium phosphate [pH 7.4], 1.2 M sorbitol) and centrifuged again. The resulting pellet was resuspended in 1.5 ml of Zymolyase buffer containing 4 mg/ml Zymolyase 20-T (MP Biomedicals Life Sciences) and incubated for 30 min at 1,000 rpm and 30°C (thermomixer). After centrifugation for 5 min at 900 x g and 4°C, spheroplasts were resuspended in 1.5 ml of ice-cold homogenization buffer (10 mM Tris/HCl [pH 7.4], 0.6 M sorbitol, 1 mM EDTA, 1 mM PMSF) and homogenized by
drawing the sample 20x up and down through a 0.8 x 22 mm blunt end needle attached to a 3-ml syringe. Cell debris was removed by centrifugation (twice for 10 min at 900 x g and 4°C). The supernatant was transferred to a fresh 2-ml reaction tube and mitochondria were pelleted by centrifugation for 5 min at 16,800 x g and 4°C. Mitochondria-enriched fractions were washed with 2 ml of ice-cold SEM buffer (250 mM sucrose, 1 mM EDTA, 10 mM MOPS [pH 7.2]) and centrifuged again for 10 min at 900 x g and 4°C followed by centrifugation of the supernatant for 5 min at 16,800 x g and 4°C. Mitochondria were resuspended in 200 µl of SEM buffer, shock-frozen in liquid nitrogen, and stored at -80°C.

Isolation of mitochondria for Western blot analyses was performed as described before with various modifications. Cells were harvested by centrifugation for 5 min at 3,000 x g, RT, washed with deionized water and incubated for 20 min at 30°C in 2 ml DTT buffer (100 mM Tris-H₂SO₄ [pH 9.4] and 10 mM DTT) per gram wet weight. After centrifugation for 5 min at 3,000 x g, RT, the cells were washed with 7 ml per gram wet weight in Zymolyase buffer (1.2 M sorbitol and 20 mM K₃PO₄ [pH 7.4]) and incubated for 45 min at 30°C in 7 ml per gram wet weight Zymolyase buffer containing 3 mg per gram wet weight Zymolyase 20-T (Nacalai Tesque, Inc.). Spheroplasts were harvested by centrifugation for 5 min at 3,000 x g, 4°C and resuspended in 7 ml per gram wet weight ice-cold homogenization buffer (0.6 M sorbitol, 10 mM Tris-HCl [pH 7.4], 1 mM EDTA and 1 mM PMSF) and homogenized using a Glass Teflon Homogenizer (20 strokes). Cell debris and nuclei were separated from the homogenate by centrifugation for 5 min at 1,500 x g, 4°C. Mitochondria were pelleted from the supernatant by centrifugation for 10 min at 12,000 x g, 4°C, resuspended in SEM buffer (250 mM sucrose, 10 mM MOPS-KOH [pH 7.2] and 1 mM EDTA), snap-frozen in liquid nitrogen and stored at -80°C until further use.

Proteolytic in-solution digestion

10 µg of protein were acetone-precipitated and resuspended in 10 µl of urea buffer (8 M urea, 75 mM NaCl, 50 mM Tris/HCl [pH 8.0], 1 mM EDTA). Cysteine residues were reduced and alkylated as described previously. (These steps were omitted for samples prepared for the analysis of incorporation efficiencies.) The urea concentration was
diluted to 4 M for LysC and 1.6 M for trypsin digestion using 50 mM NH₄HCO₃. LysC digestion was performed for 4 h at 37°C using 100 ng LysC and digestion with trypsin (200 ng) was performed overnight at 37°C. Reactions were stopped by acidification with trifluoroacetic acid (TFA; 0.5% [v/v] final concentration) and peptide mixtures were cleared by centrifugation for 5 min at 12,000 x g (RT).

**Stable isotope dimethyl labeling**

Tryptic peptides obtained from whole cell extracts were chemically labeled with ‘light’ formaldehyde (CH₂O; Sigma-Aldrich) and sodium cyanoborohydride (NaBH₃CN; Sigma-Aldrich) or the corresponding ‘heavy’, deuterated versions (CD₂O/NaBD₃CN; Sigma-Aldrich) essentially as described before⁴ with minor adaptations. In brief, StageTips were conditioned with 100% methanol (MeOH) followed by 80% (v/v) acetonitrile (ACN)/0.5% (v/v) acetic acid and 100 mM tetaethylammonium bicarbonate (TEAB). Peptides were loaded onto StageTips and washed twice with 100 mM TEAB. Labeling was performed by adding 2x 150 µl of light or heavy labeling reagents (17.5 µl 4% [v/v] CH₂O/CD₂O, 17.5 µl 0.6 M NaBH₃CN/NaBD₃CN, 70 µl 50 mM NaH₂PO₄, 245 µl 50 mM Na₂HPO₄). StageTips were washed twice with 100 mM TEAB and labeled peptides were eluted with 80% ACN/0.5% acetic acid. Solvents were evaporated and peptides were resuspended in 75 µl of 0.1% TFA. The labeling efficiency (> 95%) for light and heavy dimethyl-labeled peptides was determined by LC-MS. Heavy and light labeled peptides were mixed in equal ratios based on the overall MS intensities of individual peptide samples. Experiments were performed in three biological replicates including a label switch.

**High pH reversed-phase chromatography**

Proteolytic peptides of whole cell extracts generated from differentially light (L), medium-heavy (MH), and heavy (H) SILAC-labeled cells mixed in 1:6:1.5 (H:L:M) ratios (based on OD₆₀⁰) were fractionated by high pH reversed-phase chromatography using StageTips. To this end, peptide mixtures were loaded onto StageTips conditioned with 100% MeOH followed by 80% (v/v) ACN/0.5% (v/v) acetic acid and 0.1% TFA. Peptides were washed twice with 0.1% TFA and subsequently serially eluted into eight fractions.
with 0, 3, 6, 10, 13, 16, 40, and 72% (v/v each) ACN in 10 mM NH₄OH. Solvents were evaporated and peptides were resuspended in 30 µl of 0.1% TFA. Experiments were performed in four biological replicates.

**Liquid chromatography-mass spectrometry**

Nano-HPLC-ESI-MS/MS analyses were performed using Ultimate 3000 RSLCnano systems (Thermo Fisher Scientific, Dreieich, Germany) equipped with PepMap C18 pre-columns (Thermo Scientific; length: 5 mm; inner diameter: 0.3 mm; loading flow rate: 30 µl/min) and Acclaim PepMap analytical columns (Thermo Scientific; length: 500 mm; inner diameter: 75 µm; particle size: 2 µm; packing density: 10 nm; flow rate: 0.25 µl/min) online coupled to an LTQ Orbitrap XL or a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Peptides were washed and concentrated on precolumns and subsequently separated using a binary solvent system consisting of 4% (v/v) DMSO/0.1% (v/v) formic acid (FA; solvent A for analyses using the LTQ Orbitrap XL) or 0.1% (v/v) FA (solvent A’, Q Exactive) and 48% (v/v) MeOH/30% (v/v) ACN/4% (v/v) DMSO/0.1% FA (solvent B; LTQ Orbitrap XL) or 86% (v/v) ACN/0.1% (v/v) FA (solvent B’; Q Exactive). To determine the incorporation of SILAC amino acids and the completeness of stable-isotope dimethyl labeling, samples were analyzed on the LTQ Orbitrap XL. Peptides were eluted with a multi-step gradient starting with 25 min at 5% solvent B followed by 5 - 30% B in 65 min, 30 - 45% B in 30 min, 45 - 70% B in 35 min, 70 - 99% B in 5 min, and 99% B for 5 min. Peptides derived from stable-isotope dimethyl labeling and 2nSILAC experiments, analyzed on the Q Exactive, were eluted with 4% solvent B’ for 25 min followed by 4 - 39% B’ in 25 min, 39 - 54% B’ in 175 min, 54 - 95% B’ in 15 min, and 5 min at 95% B’. Peptides generated in triple SILAC experiments were eluted with a multi-step gradient starting with 4% solvent B’ for 5 min, followed by 4 - 40% B’ in 50 min, 40 - 95% B’ in 5 min, 95% B’ for 5 min, 95% - 4% B’ in 1 min and 4% B’ for 14 min.

Mass spectrometers were equipped with a Nanospray Flex ion source with DirectJunction (Thermo Scientific; Q Exactive) or a Finnigan Nanospray ion source with dynamic NSI probe (Thermo Scientific; LTQ Orbitrap XL) and stainless steel (Thermo Scientific) or fused silica emitters (New Objective, Woburn, USA). MS instruments were
operated in data-dependent mode. Mass spectrometric parameters for the LTQ Orbitrap XL were as follows: full MS scans were acquired in the orbitrap with a mass range of m/z 370 - 1,700, a resolution (R) of 60,000 at m/z 400, a maximum automatic gain control (AGC) target of 5 x 10^5 ions, and a maximum ion time (IT) of 500 ms. The five most intensive peptide ions (z ≥ +2) were chosen for fragmentation by low-energy collision-induced dissociation (CID) in the linear ion trap. A normalized collision energy (NCE) of 35%, an activation q of 0.25, and an activation time of 100 ms were applied. The AGC for MS/MS scans was set to 10^4, the maximum IT was 100 ms, the ion selection threshold was 2,500, and the dynamic exclusion time (DE) for previously selected precursor ions was 45 s. The Q Exactive Plus was operated with the following parameters: MS scans, m/z 375 - 1,700; R, 70,000 at m/z 200; AGC, 3 x 10^6; max. IT, 60 ms; TOP15 method for fragmentation of precursor ions (z ≥ +2) by higher-energy collisional dissociation (HCD); NCE, 28%; acquisition of fragment ion spectra at R 35,000; AGC for MS/MS scans, 10^5 ions; max. IT, 120 ms; DE, 45 s.

**MS data analysis**

Protein identification and quantification was performed using MaxQuant with its integrated search engine Andromeda (version 1.5.2.8, 1.5.3.30, or 1.5.5.1).\(^5\)\(^6\) MS/MS data were searched against the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/; downloaded September 2011) and a list of common contaminants included in the MaxQuant software. Database searches were performed with the appropriate enzymatic specificity (i.e., LysC, trypsin, or both), a maximum number of two missed cleavages, mass tolerance of 4.5 ppm for precursor and 0.5 Da (CID data) or 20 ppm (HCD data) for fragment ions, carbamidomethylation of cysteine residues as fixed modification (unless reduction and alkylation of cysteines were omitted), and oxidation of methionine and N-terminal protein acetylation as variable modifications. For the analysis of arginine-to-proline conversion, Pro6 was additionally included as variable modification. For identification, proteins were required to have at least one unique peptide with a length of at least seven amino acids. A false discovery rate of 0.01 was applied to both peptide and protein identifications. For the analysis of dimethyl-labeled proteins, dimethylLys0/dimethylNter0 and dimethylLys6/dimethylNter6...
were set as light and heavy labels; for the analysis of SILAC-labeled samples, Lys0/Arg0, Lys4/Arg6, and Lys8/Arg10 were considered as light, medium-heavy, and heavy labels. The options 'match between runs' and 'requantify' were generally enabled except for the analysis of incorporation and labeling efficiencies. Relative protein quantification was generally based on razor or unique peptides and a ratio count (i.e., SILAC peptide pairs) of ≥ 1.

The extent of Lys8 and Arg10 incorporation into the yeast proteome was determined as described before. Calculations were based on data provided in the 'evidence.txt' output file from MaxQuant, which contains all identified peptide features of the database search. For the data shown in Figure 2, the overall minimum and maximum number of peptides used to determine the incorporation of Lys8 and Arg10 for cells harvested at distinct ODs were as follows: 14,086 - 20,269 (BY4741; 2% glucose; Fig. 2a), 18,707 - 21,267 (BY4741; 2% galactose; Fig. 2b), 8,333 - 12,921 (BY4741; 3% glycerol/0.02% glucose; Fig. 2c), and 18,080 - 21,191 (W303; Fig. 2d). Data refer to experiments in which cells were grown in the presence of proline (Table S-2).

Arginine-to-proline conversion was determined (a) based on the numbers of identified Pro6-containing peptides and (b) based on the MS intensities of identified Pro6-containing peptides per dataset extracted from the MaxQuant 'evidence.txt' output file. For (a), we calculated the percentage of Pro6-containing peptide features among all identified peptide features of a dataset. For (b), we determined relative MS intensity of Pro6-containing peptides (in %) in relation to the summed MS intensity of all peptides identified per dataset. Note that the calculation of arginine-to-proline conversion using MaxQuant only considers intensities of sequenced Pro6-containing peptides and, thus, generally underestimates the conversion frequency.

The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the following data set identifiers: PXD009937 (effect of exogenous arginine and lysine on the proteome of BY4741 and W303), PXD009943 (incorporation efficiency of heavy arg/lys and arg-pro-conversion), PXD009951 (effect of exogenous proline on BY4741), PXD009980 (assessment of 2nSILAC for proteome quantification), PXD010019 (2nSILAC for the study of mito gene
deletion effect), and PXD010704 (Assessment of incorporation of heavy amino acids in mitochondrial gene deletion strains and wild-type cells).

To determine the effects of exogenous amino acids or gene deletions on the (sub)cellular yeast proteome, the mean log₂ of normalized protein abundance ratios and the corresponding p-values (two-sided Student's t-test, n=3 including a label switch) were calculated. Please note that the option 'requantify', which was enabled for the analysis of these datasets by MaxQuant, generally enables the output of SILAC ratios even if only the isotope-labeled or unlabeled variant of a peptide was present in the sample by assigning a peptide intensity for the missing counterpart from the background signals in MS spectra at the expected m/z value. Proteins exhibiting a t-test p-value of ≤ 0.05 and a Significance B p-value ≤ 0.05 were considered significantly changed in abundance. Outlier analyses using Significance B were performed using Perseus. Proteins determined to be outliers are marked in the respective supporting tables (i.e. Tables S-1, S-2, S-4, S-8, S-10, and S-11).

Data were visualized as volcano plots depicting proteins quantified with ≥ 2 ratio counts (Fig. 1a, 1b, and S-1a) or ≥ 1 ratio count (Figure 5a, 5c, and 5f). The assignment of proteins to distinct subcellular compartments was based on annotations in the Gene Ontology domain 'Cellular Component' provided by the SGD (downloaded 12/08/2016) and, for mitochondrial proteins, on the high-confidence mitochondrial proteome reported recently. GO term enrichment analyses were performed using YeastMine. GO terms with a Holm-Bonferroni corrected p-value of ≤ 0.05 were considered as over-represented. To determine the accuracy of relative protein quantification (Figure 4d), log₂ protein ratios of all replicates per mixing ratio were combined and visualized as boxplots. Boxplots were generated using the R package ggplot2.

Gel electrophoresis

SDS PAGE: Mitochondria were boiled in SDS sample buffer (60 mM Tris-HCl [pH 6.8], 2% [w/v] SDS, 10% [v/v] glycerol, 0.01% [w/v] bromphenolblue and 1% [v/v] β-mercaptoethanol) for 15 min at 70°C and subjected to 10-16.5% discontinuous tris-tricine polyacrylamide gels.
Blue native PAGE: Mitochondria were solubilized in either 1% digitonin or 1% DDM buffer (20 mM Tris-HCl [pH 7.4], 0.1 mM EDTA, 50 mM NaCl, 10% [v/v] glycerol, 1 mM PMSF and 1% [w/v] digitonin or n-Dodecyl β-D-maltoside [DDM]) and incubated on ice for 15 min. After centrifugation, blue native loading dye was added (final concentration: 50 mM ε-amino n-caproic acid, 100 mM Bis-Tris-HCl [pH 7.0] and 0.5% [w/v] Coomassie G-250) and samples were subjected to 3-10% or 5-10% discontinuous polyacrylamide gels.

_Determination of protein concentrations_

Protein concentrations were determined according to Bradford\textsuperscript{13} using bovine serum albumin as standard. Immunoblot analyses were performed following standard protocols.
### Yeast strains used in this study

| Strain               | Description       | Genotype                                      | Source or Reference            |
|----------------------|-------------------|-----------------------------------------------|--------------------------------|
| Wild-type (WT)       | BY4741            | MATa; ura3Δ0, leu2Δ0, his3Δ1, met15Δ0         | Euroscarf                      |
| Wild-type (WT)       | W303              | MATa, ura3-52, trp1Δ2, leu2-3_112, his3-11, ade2-1, can1-100 | Euroscarf (strain BMA64-1A, isogenic to W303) |
| Wild-type (WT)       | BY4741 lys2Δarg4Δ | MATa; ura3Δ0, leu2Δ0, his3Δ1, lys2Δ0, met15Δ0, arg4::kanMX4 | Morgenstern et al., 2017 (Ref. #1) |
| sdh5Δ                | BY4741 sdh5Δ      | MATa; ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, sdh5::kanMX4 | Euroscarf                      |
| phb1Δ                | BY4741 phb1Δ      | MATa; ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, phb1::kanMX4 | Euroscarf                      |
| coi1Δ*               | BY4741 coi1Δ      | MATa; ura3Δ0, leu2Δ0, his3Δ1, met15Δ0, coi1::kanMX6 | this study                     |

* The strain coi1Δ was generated by gene deletion via homologous recombination as described before.14 The kanMX6 cassette was amplified from pFA6a-kanMX6 using the following forward and reverse primers:
AAGGCAGATGAAGAGATAATATAGTAATATTTTACCTTTGAGCCGTACTAAGTATAACACG
GATCCCCGGGTAAATTAA (forward) and
GATGAATATATATACATAATACTGCAATGAAATATGGATAGGTGATTAACGCTTGGA
ATTGCAGCTCGTTAAAC (reverse).

### Antibodies used in this study

| Antigen | Dilution | Number     | Secondary antibody |
|---------|----------|------------|--------------------|
| Abf2    | 1:200 TBS-T + 5% milk | GR B2072  | anti-rabbit        |
| Atp2    | 1:50 TBS-T + 5% milk | GR863 affinity purified e4 | anti-rabbit        |
| Atp4    | 1:250 TBS-T + 5% milk | GR1970-6  | anti-rabbit        |
| Atp5    | 1:250 TBS-T + 5% milk | GR1546-4  | anti-rabbit        |
| Atp17   | 1:250 TBS-T + 5% milk | GR1968-3  | anti-rabbit        |
| Coa6    | 1:250 TBS-T + 5% milk | GR4794-6  | anti-rabbit        |
| Cor1    | 1:300 TBS + 5% milk | GR371-5   | anti-rabbit        |
| Cox1    | 1:400 TBS-T + 5% milk | GR1538-4  | anti-rabbit        |
| Cox2    | 1:250 TBS-T + 5% milk | GR1949-2  | anti-rabbit        |
| Protein | Dilution | Buffer | Code     | Antibody Type |
|---------|----------|--------|----------|---------------|
| Cox4    | 1:100    | TBS-T  | GR578-1  | anti-rabbit   |
| Cox9    | 1:250    | TBS-T  | GR3612-3 | anti-rabbit   |
| Cox12   | 1:250    | TBS-T  | GR1937-2 | anti-rabbit   |
| Cox13   | 1:250    | TBS-T  | GR1542-4 | anti-rabbit   |
|         | 1:200    |        | GR1543-1 |               |
| Cyt1    | 1:500    | TBS-T  | GR540-3  | anti-rabbit   |
| Goat IgG| 1:50000  | TBS-T  | Peroxidase antibody, rabbit (Sigma-Aldrich Corp.; A8919) | - |
| Mcr1    | 1:250    | TBS-T  | GR614-3  | anti-rabbit   |
| Mdl1    | 1:200    | TBS    | GR1518-7 | anti-rabbit   |
| Mdm38   | 1:200    | TBS    | GR343-1  | anti-rabbit   |
| Mic10   | 1:500    | TBS-T  | GR3343-2 | anti-rabbit   |
| Nde1    | 1:250    | TBS-T  | GR3026-6 | anti-rabbit   |
| Om45    | 1:100    | TBS-T  | GR1391-4 | anti-rabbit   |
| Phb2    | 1:200    | TBS-T  | B295-3   | anti-rabbit   |
| Por1    | 1:500    | TBS-T  | GR3622-3 | anti-rabbit   |
| Qcr6    | 1:250    | TBS    | GR1054-6 | anti-rabbit   |
| Qcr8    | 1:200    | TBS    | GR1038-1 | anti-rabbit   |
| Rip1    | 1:500    | TBS-T  | GR542-3  | anti-rabbit   |
|         |          |        | GR543-5  |               |
| Sdh1    | 1:1000   | TBS-T  | GR1849-3 | anti-rabbit   |
| Sdh3    | 1:250    | TBS-T  | GR2434-5 | anti-rabbit   |
| Sdh4    | 1:2000   | TBS-T  | GR1855-3 | anti-rabbit   |
| Tim23   | 1:500    | TBS-T  | GR133-9  | anti-rabbit   |
| Tom70   | 1:500    | TBS-T  | GR657-4  | anti-rabbit   |
| Yme1    | 1:400    | TBS-T  | GR1455-3 | anti-rabbit   |
| Yta12   | 1:250    | TBS-T  | GR1438-4 | anti-rabbit   |
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