Impact of lipopolysaccharides on cultivation and recombinant protein expression in human embryonal kidney (HEK293) cells

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Table S1. Transcriptomic data for gene products involved in LPS induced signaling. The transcriptomic data (given as transcripts per kilobase millions, TPM) for the original HEK cell line compared to the Freestyle 293F cell line (Gibco). The original HEK293 cell line is an adherent cell line whereas the FreeStyle 293 F cell line has been adapted to suspension growth in Freestyle 293 medium. In addition to proteins involved in LPS induced signaling transcriptomic data two housekeeping proteins, Hsp90 and GAPDH, were extracted as well for comparison reasons.

| Proteins involved in TLR2/4 mediated signaling (Ciesielska et al., 2020; Zamytina and Heine, 2020; Lannoy et al., 2021) | Function and interaction partner (https://www.uniprot.org) | ENSEMBL Gene ID (http://www.ensembl.org) | Transcriptomic data (TPM values) (Malm et al. 2020) |
|---|---|---|---|
| | HGN CIS name (https://www.genenames.org) | UniProt ID | Original HEK293 | FreeStyle 293-F |
| | | | (1) | (2) | (3) | (1) | (2) | (3) |
| LBP | lipopolysaccharide binding protein | P18428 | ENSG00000123988 | 0,120979 | 0,0817696 | 0,221325 | 0,0884682 | 0,310747 | 0,127904 |
| CD14 | CD14 molecule | P08571 | ENSG00000170458 | 2,572599 | 0,64781894 | 1,268851 | 0,0924843 | 0,113852 | 0,553214 |
| MD2 (LY96) | lymphocyte antigen 96 | Q9Y6Y9 | ENSG00000154589 | 0,186414 | 0,18295 | 0 | 0,204613 | 0 | 0 |
| MYD88 | MYD88 innate immune signal transduction adaptor | Q99836 | Involved in the Toll-like receptor and IL-1 receptor signaling. Acts via IRAK1, IRAK2, IRF7 and TRAF6. | ENSG00000172936 | 5,867173 | 6,48120238 | 7,469238 | 3,6214196 | 3,72571165 | 3,018348 |
| TIRAP (MAL) | TIR domain containing adaptor protein | PS8753 | Adapter involved in TLR2 and TLR4 signaling. Acts via IRAK2 and TRAF6. | ENSG00000150455 | 10,21065 | 7,63318 | 9,232315 | 8,260648 | 9,846641 | 9,492699 |
| IRAK1 | interleukin 1 receptor associated kinase 1 | P51617 | Recruited by MYD88 to the receptor-signaling complex upon TLR activation. Association with MYD88 leads to IRAK1 phosphorylation by IRAK4 and subsequent autophosphorylation and activation. | ENSG00000184216 | 197,30994 | 168,67898 | 199,075793 | 139,432152 | 128,559078 | 128,108009 |
| IRAK2 | Interleukin-1 receptor-associated kinase-like 2 | Q43187 | Binds to the IL-1 type 1 receptor following IL-1 engagement. | ENSG00000134070 | 1,51243 | 1,33363 | 1,85116 | 2,11621 | 2,99393 | 2,79266 |
| IRAK3 (IRAK-M) | interleukin 1 receptor associated kinase 3 | Q9Y616 | Inhibits dissociation of IRAK1 and IRAK4 from the Toll-like receptor signaling complex by either inhibiting the phosphorylation of IRAK1 and IRAK4 or stabilizing the receptor complex. | ENSG0000009376 | 0,0430162 | 0,00849972 | 0,1180103 | 0,0181713 | 0,06629376 | 0,0264974 |
| IRAK4 | interleukin 1 receptor associated kinase 4 | Q9NWZ3 | Involved in Toll-like receptor (TLR) and IL-1R signaling pathways. Is rapidly recruited by MYD88 to the receptor-signaling complex upon TLR activation to form the Myddosome together with IRAK2. | ENSG00000198001 | 10,032468 | 6,544035 | 8,22449791 | 6,63711875 | 7,82464692 | 7,611479 |
| TRAF6 | TNF receptor-associated factor 6 | Q9Y4K3 | E3 ubiquitin ligase. Conjugates proteins such as IKKbG, IRAK1, AKT1, AKT2. | ENSG00000175104 | 14,66179 | 10,41034 | 12,48567 | 9,03194 | 7,82369 | 9,43827 |
| TICAM1 (TRIF) | toll like receptor adaptor molecule 1 | Q8IU6C | Adapter used by TLR3, TLR4 (through TICAM2) and TLR5 | ENSG00000127666 | 6,393694 | 5,91461 | 10,2914 | 4,88021 | 4,72534 | 5,13877 |
| TICAM2 (TRAM) | toll like receptor adaptor molecule 2 | Q86XR7 | In TLR4 signaling, physically bridges TLR4 and TICAM1. In TLR2 signaling, physically bridges TLR2 and MYD88 | ENSG00000243414 | 3,77869 | 3,7609 | 4,23024 | 0,23103 | 0,31033 | 0 |
| TRAM1 | translocation associated membrane protein 1 | Q15629 | Required for the translocation of secretory proteins across the ER membrane | ENSG0000067167 | 100,102185 | 91,121329 | 125,319563 | 74,689093 | 66,390675 | 64,3603992 |
| IRF7 | Interferon regulatory factor 7 | Q92985 | Key transcriptional regulator of type I interferon (IFN)-dependent immune responses. Activated by TLR signaling. | ENSG00000185507 | 0 | 1,443596 | 1,589136 | 0,239734 | 0,7997894 | 0,440245 |
| Protein Name | Description | Gene ID | ... |
|--------------|-------------|---------|-----|
| TLR1 | toll like receptor 1 | Q15399 | Recognition of diacylated and triacylated lipopolysaccharides. Acts via MYD88 and TRAF6. | ENSG000000174125 |
| TLR2 | toll like receptor 2 | O60603 | Recognition of lipoproteins or lipopolysaccharides. Can also interact with TLR4 and TLR6. Acts via MYD88 and TRAF6. | ENSG000000137462 |
| TLR3 | toll like receptor 3 | O15455 | Nucleotide-sensing, double-stranded RNA. Acts via TICAM1 | ENSG000000164342 |
| TLR4 | toll like receptor 4 | O00206 | Coreceptor for bacterial lipopolysaccharide. Can also interact with TLR2 and TLR6. In concert with LBP binds to monomeric LPS and delivers it to the TLR4/MD2 complex. | ENSG000000136869 |
| TLR5 | toll like receptor 5 | O60602 | Recognizes small molecular motifs named pathogen-associated molecular pattern (PAMPs) expressed by pathogens and microbe-associated molecular patterns (MAMPs) usually expressed by resident microbe. Acts via MYD88 and TICAM1. | ENSG000000187554 |
| TLR6 | toll like receptor 6 | Q9Y2C9 | Specifically recognizes diacylated and, to a lesser extent, triacylated lipopolysaccharides. Can also interact with TLR2 and TLR4. Acts via MYD88 and TRAF6. | ENSG000000174130 |
| TLR7 | toll like receptor 7 | Q9NYK1 | Recognition of uridine-containing single strand RNAs (ssRNAs) of viral origin or guanosine analogs. Acts via MYD88. | ENSG000000196664 |
| TLR8 | toll like receptor 8 | Q9NR97 | Recognition of RNA degradation products specific to microorganisms that are initially processed by RNASE12. Acts via MYD88. | ENSG000000101916 |
| TLR9 | toll like receptor 9 | Q9NR96 | Recognition of molecular patterns specific to microorganisms. Nucleotide-sensing TLR activated by unmethylated cytidine-phosphate-guanosine (CPG) dimucleotides. Acts via MYD88 and TRAF6. | ENSG000000239732 |

**Proteins involved in intracellular LPS sensing and pyroptosis** (Rathinam et al., 2019; Downs et al., 2020)

| Protein Name | Description | Gene ID | ... |
|--------------|-------------|---------|-----|
| Caspase 4 | Inflammatory caspase that acts as an essential effector of NLRP3 inflammasome-dependent casp1 activation and IL1B and IL18 secretion in response to non-canonical activators, such as UVB radiation, cholera enterotoxin subunit B and cytosolic LPS | ENSG000000196954 |
| Caspase 5 |...| ENSG000000137757 |
| GSDMD | Precursor of a pore-forming protein that plays a key role in host defense against pathogen infection and danger signals | ENSG000000104518 |

**Housekeeping proteins** (Picard, 2002; Sikand et al., 2012)

| Protein Name | Description | Gene ID | ... |
|--------------|-------------|---------|-----|
| HSP90 alpha | Molecular chaperone that promotes the maturation, structural maintenance and proper regulation of specific target proteins involved for instance in cell cycle control and signal transduction. | ENSG000000080824 |
| GAPDH | Glyceraldehyde-3-phosphate dehydrogenase | ENSG000000111640 | Has glyceraldehyde-3-phosphate dehydrogenase activity, required for glycolysis. | ENSG000000111640 |

**Glycolysis**

Has glyceraldehyde-3-phosphate dehydrogenase activity, required for glycolysis.
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Figure S1. Cell count (A) and antibody titers (B) for experiment 2. First time point is the day after cell culture splitting (d1). Antibody titers were measured directly in the culture supernatants.
**Figure S2.** Cell count (A) and antibody titers (B) for experiment 3. First time point is the day after cell culture splitting (d1). Antibody titers were measured directly in the culture supernatants.
Figure S3. Cell size for the three different and independent experiments (A)-(C).
**Figure S4.** Cell aggregation of the cell cultures for the three different experiments (A)-(C). Cells were classified as “aggregates” when five or more cells stick together as interpreted by the NucleoView NC-200 software.

If no value is given a reliable measurement was not possible due to increasing cell clumping and cell lysis.