Mouse TLR3 ELISA Kit

Enzyme Immunoassay for the quantification of Mouse TLR3 in Mouse Serum, plasma and cell culture supernatants.

Catalog number: ARG82241

Package: 96 wells

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INTRODUCTION

The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs are highly conserved from Drosophila to humans and share structural and functional similarities. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The various TLRs exhibit different patterns of expression. This receptor is most abundantly expressed in placenta and pancreas, and is restricted to the dendritic subpopulation of the leukocytes. It recognizes dsRNA associated with viral infection, and induces the activation of NF-kappaB and the production of type I interferons. It may thus play a role in host defense against viruses. Use of alternative polyadenylation sites to generate different length transcripts has been noted for this gene. [provided by RefSeq, Jul 2008] Key component of innate and adaptive immunity. TLRs (Toll-like receptors) control host immune response against pathogens through recognition of molecular patterns specific to microorganisms. TLR3 is a nucleotide-sensing TLR which is activated by double-stranded RNA, a sign of viral infection. Acts via the adapter TRIF/TICAM1, leading to NF-kappa-B activation, IRF3 nuclear translocation, cytokine secretion and the inflammatory response. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique. An antibody specific for TLR3 has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TLR3 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for TLR3 is added to each well and incubate to bind to TLR3 captured by the first antibody. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of TLR3 bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of TLR3 in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	1 X 96 well plate	4°C
Standard	3 X 4 ng/vial (Lyophilized)	4°C
Biotin-antibody conjugate concentrate	1 vials (lyophilized)	4°C
HRP-Streptavidin conjugate concentrate	1 vial (55 µl)	4°C
20X PBS	25 ml	4°C
20X Assay Buffer	20 ml	4°C
TMB substrate	10.5 ml (Ready to use)	4°C (Protect from light)
STOP solution	5.5 ml (Ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 540-570 nm as the reference wave length)
- Pipettes and pipette tips
- Deionized or distilled water
- Sterile 1 x PBS
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
- Briefly spin down (6000Xg for 1 min) the Standards, Biotin-antibody conjugate and HRP-streptavidin conjugate before use.
- If crystals are observed in the 20X Assay Buffer, warm to RT until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- A standard curve should be generated for each set of samples assayed. Thorough mixing of standards at each of dilution steps is critical to acquire a normal standard curve.
- Brief vortex samples and diluted standards for 10 sec to mix well before add to the 96 well plate.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Do not let strips dry, as this will inactivate active components in wells.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- HRP Conjugate contains enzyme, DO NOT mass up with Detection Antibody.

- The Stop Solution is an acid solution, handle with caution.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X PBS: Dilute 20X PBS into deionized distilled water to yield 1X PBS.
- 1X Assay Buffer: Dilute 20X Assay Buffer into 1X PBS to yield 1X Assay buffer. The diluted 1X Assay Buffer can be stored at 4°C.
- 1x Biotin-antibody Conjugate: The lyophilized Biotin-antibody conjugate could be stored at 4°C to -20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the Biotin-antibody Conjugate with 200 µl of sterile 1 x PBS, vortex for 30 sec and keep the antibody in the vail for 5 min to completely dissolve. Centrifuge the vial for 1 min at 6000 x g before opening. Aliquot and store the antibody stock at -20°C until use. Avoid repeated freeze-thaw cycles.

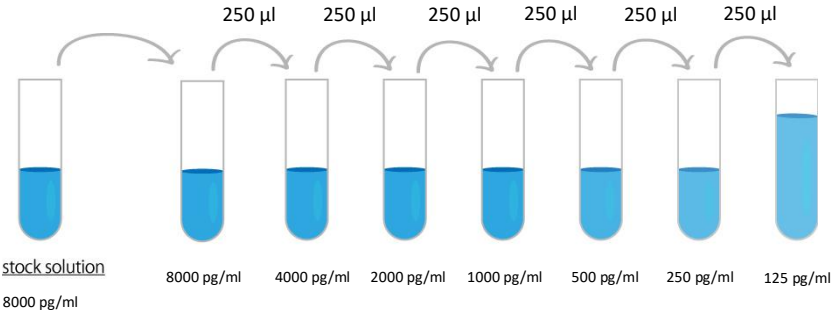
  If the entire 96-well plate is used, dilution of the 200 µl of concentrated Biotin-Conjugate solution with 10.5 ml 1X PBS to yield 1X Biotin-antibody Conjugate working solution.
- 1X HRP-streptavidin conjugate: Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. The stock vial includes 55 µl of HRP-streptavidin concentrate. Please confirm if the vial contains 55 µl of HRP-streptavidin concentrate before further dilution. If it is less than 55 µl, add sterile 1X PBS to reach 55 µl and vortex briefly for 10 sec. Make a 1:200 dilution of the concentrated HRP-streptavidin solution with 1X PBS (If the entire 96-well plate is used, add 53 µl of concentrated HRP-streptavidin solution into 10.5 ml of 1X PBS and mix thoroughly prior to the assay). The rest of undiluted HRP-streptavidin Conjugate can be stored at 4°C for up to 3 months. DO NOT FREEZE.

- **Sample**: Samples should be diluted with equal volume of 1 x Assay Buffer and vortex for 1 min prior to assay. If the initial assay found samples contain TLR3 higher than the highest standard, the samples can be diluted with 1 x Assay Buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. The sample must be well mixed with 1 x Assay Buffer before assay.

  (It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: The non-reconstituted standard can be stored at 4°C or-20°C for up to 3 months. Centrifuge the vial for 1 min at 6000 x g to spin down the material prior to open the vial. Reconstitute the standard with **0.5 ml 1 x Assay Buffer** to yield a stock concentration of 8000 pg/ml. Brief vortex the vials for 30 sec and keep the standard stock in the vail for 5 min to completely dissolve. Make sure the standard is dissolved completely and then centrifuge the vial for 1 min at 6000 x g before making serial dilutions. Aliquot and store the reconstituted standard at -20°C for up 2 days.

  The 1 x Assay Buffer serves as zero standard (0 pg/ml), and the rest of the standard serial dilution can be diluted with 1X Assay Buffer as according to the suggested concentration below: **8000 pg/ml, 4000 pg/ml, 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml**. Brief vortex the vials for 30 sec for each standard dilution steps to mix well.



Dilute TLR3 standard as according to the table below:

Standard	TLR3 Conc. (pg/ml)	µl of 1X Assay Buffer	µl of standard
S7	8000 pg/ml	0	500 (8000 pg/ml Stock)
S6	4000 pg/ml	250	250 (S7)
S5	2000 pg/ml	250	250 (S6)
S4	1000 pg/ml	250	250 (S5)
S3	500 pg/ml	250	250 (S4)
S2	250 pg/ml	250	250 (S3)
S1	125 pg/ml	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be **assayed in duplicates.**

1. Lift the plate cover from the top left and cover the wells that are not used. Brief vortex and then spin down the standards and samples for 10 sec to mix completely before applying to the plate.
2. Add **100 µl** of standards, samples and zero controls (1X Assay Buffer) **in duplicates** into wells. Incubate for **2 hours** at **room temperature**.
3. Aspirate each well and wash, repeating the process once for a **total two washes**. Wash by filling each well with **1× Assay Buffer** (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Assay Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µl** of **1x Biotin-antibody Conjugate working solution** to each well. Cover the plate and incubate **1 hour** at **room temperature**.
5. Aspirate each well and **wash as step 3**.
6. Add **100 µl** of **1X HRP-Streptavidin solution** to each well. Cover wells and incubate for **20 minutes** at **room temperature** in dark.
7. Aspirate each well and **wash as step 3** but wash for **a total four washes**.
8. Add **100 µl** of **TMB Substrate Solution** to each well. Incubate for **5-30 minutes** (depending on signal) at **room temperature** in dark.
9. Add **50 µl** of **Stop Solution** to each well. Gently tap the plate to ensure thorough mixing.
10. Read the OD with a microplate reader at **450 nm** immediately. (Optional:

it is recommended to detect background signal by reading the signal at 540-570 nm as reference wavelength).

CALCULATION OF RESULTS

1. (Optional) Subtract the absorbance of the value reading at 540-570 nm from the value reading at 450 nm.

2. Calculate the average absorbance values for each set of standards, controls and patient samples.

3. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

4. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

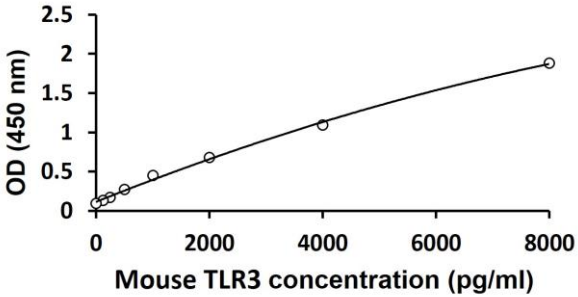
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

7. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Mouse TLR3 ranged from 125- 8000 pg/ml. The mean MDD was 62.5 pg/ml.

Specificity

This assay recognizes natural and recombinant Mouse TLR3. No significant cross-reactivity or interference with the factors below was observed:

Recombinant Mouse proteins were tested and exhibited no cross-reactivity or interference: Adiponectin, BD2, IL1 beta, IL2, IL4, IL5, IL6, IL8, IL10, IFN gamma, NLRP3, TLR1, TLR2, TLR4, TNF alpha.

Intra-assay and Inter-assay precision

The CV values of intra-assay was 6% and inter-assay was 11%.