



Tlr4 Chemi-Luminescent ELISA Kit (Mouse) (OKCD03981) Instructions for use

For the quantitative measurement of Tlr4 in serum, plasma, tissue homogenates and other biological fluids.

This product is intended for research use only.

Lot to lot kit variations can occur. Refer to the manual which has been provided with the kit.

Contents

1. Background.....	2
2. Assay Summary.....	3
3. Storage and Stability.....	3
4. Kit Components	3
5. Precautions	4
6. Required Materials Not Supplied	4
7. Technical Application Tips	4
8. Reagent Preparation.....	5
9. Sample Preparation	7
10. Assay Procedure.....	8
11. Calculation of Results	9
12. Typical Expected Data	9
13. Technical Resources	11

1. Background

Principle

Aviva Systems Biology Tlr4 Chemi-Luminescent ELISA Kit (Mouse) (OKCD03981) is based on standard sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific for Tlr4 has been pre-coated onto a 96-wellplate (12 x 8 Well Strips). Standards or test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for Tlr4 is added, incubated and followed by washing. Avidin-Peroxidase Conjugate is then added, incubated and unbound conjugate is washed away. An enzymatic reaction is produced through the addition of a luminol substrate which is catalyzed by the HRP to produce light emission. The light emission is read by a luminometer (or photo-multiplier equipped instrument) and the intensity of the emitted light is proportional to the amount of sample Tlr4 captured in well.

Background

Cooperates with LY96 and CD14 to mediate the innate immune response to bacterial lipopolysaccharide (LPS) . Acts via MYD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response . Also involved in LPS-independent inflammatory responses triggered by free fatty acids, such as palmitate. In complex with TLR6, promotes sterile inflammation in monocytes/macrophages in response to oxidized low-density lipoprotein (oxLDL) or amyloid-beta 42. In this context, the initial signal is provided by oxLDL- or amyloid-beta 42-binding to CD36. This event induces the formation of a heterodimer of TLR4 and TLR6, which is rapidly internalized and triggers inflammatory response, leading to the NF-kappa-B-dependent production of CXCL1, CXCL2 and CCL9 cytokines, via MYD88 signaling pathway, and CCL5 cytokine, via TICAM1 signaling pathway, as well as IL1B secretion. Binds electronegative LDL (LDL-) and mediates the cytokine release induced by LDL- .

General Specifications

General Specifications	
Range	0.156 - 10 ng/mL
LOD	< 0.048 ng/mL (Derived by linear regression of OD ₄₅₀ of the Mean Blank + 2xSD)
Specificity	Mouse Toll-like receptor 4 UniProt ID: Q9QUK6 GeneID: 21898 Target Alias: lipopolysaccharide response, Lps, Ly87, Ran/M1, Rasl2-8, Toll-like receptor 4
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at 4°C for 1 month or -20°C for 6 months, noted exceptions below. Do not use past expiration date.

4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
Anti-Tlr4 Microplate	96 Wells (12 x 8 Well strips)	4°C for 1 Month or -20°C for 6 Months
Tlr4 Lyophilized Standard	2 x 20 ng	
100X Biotinylated Tlr4 Detector Antibody	1 x 120 µL	
100X Avidin-HRP Conjugate	1 x 120 µL	
Standard Diluent	1 x 20 mL	
Detector Antibody Diluent	1 x 12 mL	4°C for 6 Month
Conjugate Diluent	1 x 12 mL	
30X Wash Buffer	1 x 20 mL	
100X Luminol Substrate	1 x 2 mL	
Substrate Diluent	1 x 20 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.

- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

- Luminometer or photo-multiplier tube (PMT) equipped microplate reader capable of the following parameters: lag time 30.0 seconds, read time 1.0 seconds per well.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 μ L through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.
- 37°C Incubator (optional)

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting light intensity measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to 37°C prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 μ L is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates, fibrin strands or bilirubin, or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- Luminol Substrate is easily contaminated and labile. Handle carefully and protect from light.