



Bovine toll-like receptor 2, TLR2 ELISA kit

Catalog No. E0663b

(96 tests)

Operating instruction

**FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!**

Intended use

This immunoassay kit allows for the use in vitro quantitative determination of bovine toll-like receptor 2, TLR2 concentrations in cell culture supernates, tissue homogenates and other biological fluids.

Introduction

TLR-2 is the name for a biomolecule, which plays a role in the bovine immune system. It is a membrane protein, a receptor, which sits on the surface of certain cells and which can recognize native or foreign substances, and passes on appropriate signals to the cell and/or the nervous system. The TLR-2 discussed here is member of a large family of homologous Toll-like receptors (TLRs).

TLR-2 is a membrane receptor found at the surface of immune system cells that recognises many bacterial, fungal, viral and endogenous substances. Phagocytosis of bound materials takes place in endosome/phagosome and a cellular activation, so that the elements of the innate immune system take over such as macrophages, PMN and dendritic cells tasks of the nonspecific immune defense, B1a and form MZB first anti-bodies and uses in the process the specific anti-body formation. Here cytokine is involved e.g. Tumor necrosis factor-alpha (TNF α) and different interleukins (IL-1alpha, IL-1beta, IL-6, IL-8, IL-12). Before TLRs were discovered, some the materials specified below were grouped under the term so-called "Moduline". By that rather Th1 appropriate cytokine pattern is seen in most experimental models an immune deviation this way, away of the Th2-expression. Conjugates are developed as vaccines or already used without a priori knowledge.

One only 2006 recognized characteristic is the Expression of the TLR-2 on the Tregs (special form of the T-cells), which is brought equally to TCR determined proliferation and functional inactivity. Thereby, a disinhibition of the early inflammation phase and the specific anti-body formation is reached. After reduction of the exciter number many exciter-specific Tregs are present, which, now without TLR-2-Signal, become active and the specific like the inflammatory immune reaction to restrain (see also TGF beta, Interleukin 10). Older literature, which attributes a direct immune stimulation effect over TLR-2 to a given substance, must be interpreted under circumstances that the used TLR-2-knockouts has regularly quite few Tregs.

Functionally relevant polymorphism is described, which reduced to a function restriction and thus usually survival rate with infections/sepsis with Gram-positive bacteria leads in particular.

The signal transduction is represented in the article toll-like receptor.

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TLR-2 is expressed on microglia, Schwann cells, monocytes, macrophages, dendritic cells, polymorphonuclear leukocytes, B-cells (B1a, MZB, B2), T-cells including regulatory T cells (CD4, CD25). TLR-2 is likewise in the epithelium of the bronchial tube and the alveoli.

Test principle

The microtiter plate provided in this kit has been pre-coated with an antibody specific to TLR2. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for TLR2 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain TLR2, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm. The concentration of TLR2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 x 20ml
Assay Diluent A	1 x 10ml
Assay Diluent B	1 x 10ml
Detection Reagent A	1 x 120ul
Detection Reagent B	1 x 120ul
Wash Buffer (25 x concentrate)	1 x 30ml
Substrate	1 x 10ml
Stop Solution	1 x 10ml
Plate sealer for 96 wells	1 x 5



Sample collection and storage

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at $\leq -20^{\circ}\text{C}$. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$.

Cell culture supernates and other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Note: Tissue homogenates and cell culture supernatant samples to be used within 7 days may be stored at $2-8^{\circ}\text{C}$, otherwise samples must stored at -20°C (≤ 3 months) or -80°C (≤ 6

months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.

It is recommended that all samples be assayed in duplicate.

DO NOT USE HEAT-TREATED SPECIMENS.

Limitations of the procedure

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1. The kit should not be used beyond the expiration date on the kit label.
2. Do not mix or substitute reagents with those from other lots or sources.
3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Reagent preparation

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 30 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions. The undiluted standard serves as the high standard (30 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL).

Detection Reagent A and B - Dilute to the working concentration specified on the vial label using Assay Diluent A and B (1:100), respectively.

Assay procedure

Allow all reagents to reach room temperature. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.

1. Add 100 uL of **Standard**, **Blank**, or **Sample** per well. Cover with the Plate sealer. Incubate for 2 hours at 37° C.
2. Remove the liquid of each well, don't wash.
3. Add 100 uL of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37°C. **Detection Reagent A** working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 uL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 uL of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37° C.
6. Repeat the aspiration/wash as in step 4.
7. Add 90 uL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37°C. Protect from light.
8. Add 50 uL of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Specificity

This assay recognizes recombinant and natural bovine TLR2. No significant cross-reactivity or interference was observed.

Sensitivity

The minimum detectable dose of bovine TLR2 is typically less than 0.12 ng/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero.

Detection Range

0.47-30 ng/mL. The standard curve concentrations used for the ELISA's were 30 ng/mL, 15 ng/mL, 7.5 ng/mL, 3.75 ng/mL, 1.88 ng/mL, 0.94 ng/mL, 0.47 ng/mL.

Important Note:

1. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of all standards and specimens, although not required, is recommended.
5. When mixing or reconstituting protein solutions, always avoid foaming.
6. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
7. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
8. Do not substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average

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zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the TLR2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Storage of test kits and instrumentation

1. Unopened test kits should be stored referring to the package label for frequent use, and stored at -20°C for long time storage. The microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at $2-8^{\circ}\text{C}$ in their pouch with the desiccant provided.
4. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.
5. Use fresh disposable pipette tips for each transfer to avoid contamination.
6. Substrate Solution is easily contaminated. If bluish prior to use, do not use.

Precaution

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

