DevKit Duo Plus Human TLR5 ELISA Development Kit

Catalog Number: LS-F71809

Description

This kit includes an anti-TLR5 antibody pair (unlabeled capture antibody and biotinylated detection antibody) as well as a calibration standard, intended for use in immunoassay development, such as ELISA. Approximately 5 standard 96-well Sandwich ELISA plates with a range of 0.312–20 ng/ml can be prepared with the supplied reagents, based upon the recommended concentrations and protocols. However, individual results may vary depending upon the researcher's specific assay protocol and other variables.

Target

Human TLR5 (TLR5, toll-like receptor 5, TIL3, Toll-like receptor 5, SLEB1)

Species Reactivity

Human

Supplied Materials

- **Capture Antibody:** 400 µg of unlabeled Mouse anti-TLR5 polyclonal antibody in 400 µl of buffer containing 50% Glycerol, 0.1% Sodium Azide. The recommended working dilution of this antibody for use in ELISA is 1:[supplied at the time of shipping], however, the optimal dilution should be determined experimentally by the researcher.

- **Biotinylated Detection Antibody:** 100 µg of biotinylated Rabbit anti-TLR5 polyclonal detection antibody in 800 µl of buffer containing 50% Glycerol, 0.1% Sodium Azide. The recommended dilution of this antibody for use in ELISA is 1:[supplied at the time of shipping], however, the optimal dilution should be determined experimentally by the researcher.

- **Calibration Standard (Lyophilized):** 10 µg of Human TLR5 recombinant protein for use in the generation of a Standard Curve against which the concentration of protein in unknown samples can be determined.

SAMPLE ONLY

Storage

Store at 2°C to 8°C for 1 month. Aliquot and store at -20°C for 1 year.

Applications

The supplied antibody pair is intended for use in the development of immunoassays such as ELISA. In the following pages are recommendations for ELISA plate preparation and running a traditional Sandwich ELISA.

Restrictions

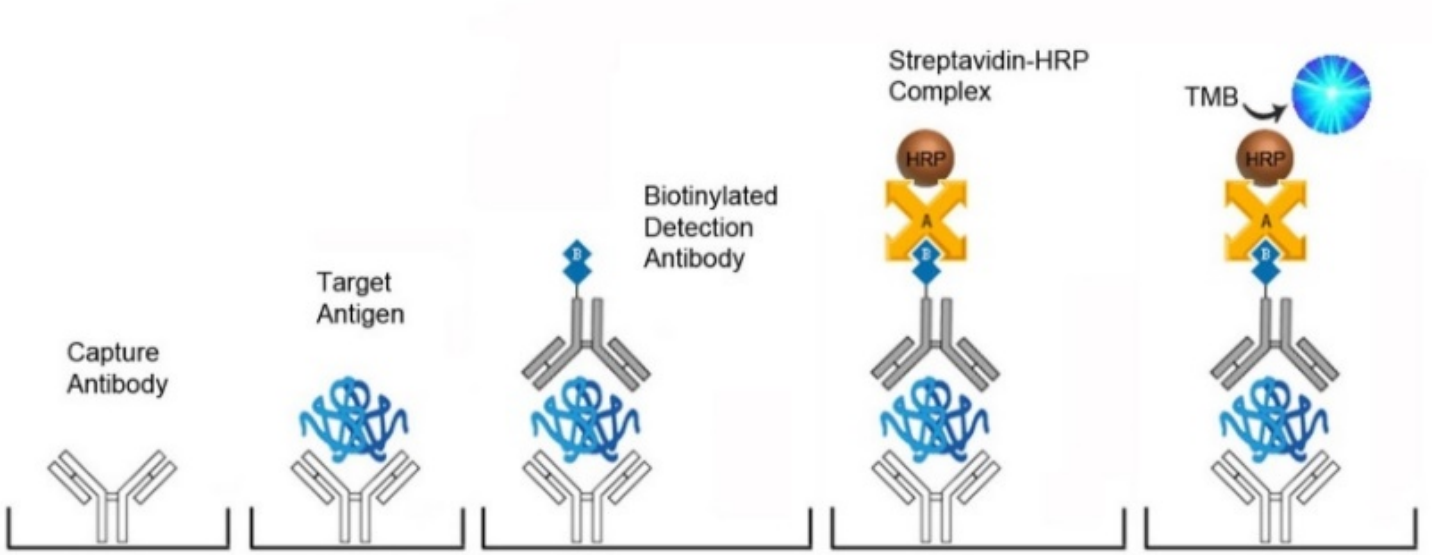
Not for resale. For research use only. Not approved for use in humans or for clinical diagnosis.

Recommended Protocols

The following protocol for plate preparation and ELISA are recommendations only. Researchers are highly encouraged to review the **LSBio ELISA Development Kit Guide** (LSBio.com/resources/devkitfaq) to order to determine which protocol and reagents are optimal for their particular application.

Assay Principle

The following plate preparation and ELISA protocol are based upon a traditional Sandwich ELISA principle. Each well of a microtiter plate is coated with a target-specific capture antibody. Standards or samples are then added to the wells and the target antigen binds to the capture antibody. Unbound Standard or sample is washed away. A biotin-conjugated detection antibody is then added which binds to the captured antigen. Unbound detection antibody is washed away. An Avidin-Horseradish Peroxidase (HRP) conjugate is then added which binds to the biotin. Unbound Avidin-HRP conjugate is washed away. A TMB substrate is then added which reacts with the HRP enzyme resulting in color development. A sulfuric acid stop solution is added to terminate color development reaction and then the optical density (OD) of the well is measured at a wavelength of 450 nm ± 2 nm. An OD standard curve is generated using known antigen concentrations; the OD of an unknown sample can then be compared to the standard curve in order to determine its antigen concentration.



Recommended Materials or Solutions

The following materials are recommended but not supplied. Each of these reagents are discussed in detail in the **LSBio ELISA Development Kit Guide** (LSBio.com/resources/devkitfaq)**.** All solutions should be at ambient temperature prior to use.

- 96-Well EIA/RIA Stripwell Plate (Cat. # LS-M42)
- ELISA Plate Sealing Covers (Cat. # LS-M45)
- ELISA Kit Support Pack 1 (Cat. # LS-K1087)

  The LS-K1087 ELISA Kit Support Pack 1 contains all of the buffers and reagents needed to prepare plates and run the ELISA as recommended. Included are the following:

  - Coating Buffer
  - Blocking Buffer
  - Standard Diluent
  - Wash Buffer
  - Detection Antibody Diluent
  - Streptavidin-HRP Diluent
  - Streptavidin-HRP Conjugate
  - TMB Substrate Solution
  - Stop Solution

Read the following protocols thoroughly and prepare all reagents before beginning an experiment. All reagents and materials should be equilibrated to room temperature (approximately 37°C) prior to the experiment.

Plate Preparation Protocol

1. Determine the volume of diluted Capture Antibody needed to prepare the intended number of wells/plates (100 µl per well/~10 ml per 96-well plate). Immediately prior to use prepare the required volume by diluting the supplied Capture Antibody [supplied at the time of shipping] times with Coating Buffer.
2. Add 100 µl of diluted Capture Antibody to each well, cover with a plate sealer, and incubate for 2 hours at 37°C or overnight at 4°C.
3. Aspirate the contents of each well and wash with 350 µl of Wash Buffer. Allow the wash buffer to sit for 1-2 minutes before aspirating. Invert the plate on a paper towel to remove residual buffer. Do not allow the plate to dry completely at any time.
4. Add 200 µl of Blocking Buffer to each well, cover with a plate sealer, and incubate for 90 minutes at 37°C.
5. Repeat the aspirate/wash process as outlined in step three.
6. Use the prepared plate immediately or cover with a plate sealer and store at -20°C in a sealed bag containing a desiccant pack. Stability during storage should be determined by the customer.

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ELISA Protocol

7. **Calibration Standard Preparation**: Reconstitute the supplied Standard with 1.0 ml of Standard Diluent. Prepare a 7-step 2-fold serial dilution series of the Standard using Standard Diluent to produce a range of 20 to 0.312 ng/ml. Use Standard Diluent alone as a Blank. Standard dilution curves should be run in duplicate. **See the LSBio ELISA Development Kit Guide** (LSBio.com/resources/devkitfaq) for more information about standard selection and preparation.
8. **Sample Preparation:** Researchers must determine the optimal dilution for their particular sample type. It is recommended that a dilution series of each sample type be run in order to identify the optimal dilution. Samples should be diluted with the same diluent as the Standards and Blank and run in duplicate or triplicate.
9. Add 100 µl of Standard, Blank, or Sample per well, cover with a plate sealer, and incubate for 60 minutes at 37°C.
10. Repeat the aspirate/wash process as outlined in step three.
11. Determine the volume of diluted Biotinylated Detection Antibody needed (100 µl per well/~10 ml per 96-well plate). Prior to use, prepare the required volume by diluting the supplied Biotinylated Detection Antibody [supplied at the time of shipping] times with Detection Antibody Diluent.
12. Add 100 µl of diluted Biotinylated Detection Antibody to each well, cover with a plate sealer, and incubate 60 minutes at 37°C.
13. Repeat the aspirate/wash process three times as outlined in step three.
14. Add 100 µl of diluted HRP-Streptavidin solution to each well, cover with a plate sealer, and incubate for 30 minutes at 37°C.
15. Repeat the aspirate/wash process five times as outlined in step three.
16. Add 90 µl of TMB Substrate Solution to each well, cover with a new plate sealer, and incubate 10 to 20 minutes at 37°C. Protect from light and monitor periodically until optimal color development has been achieved.
17. Add 50 µl of ELISA Stop Solution to each well. The Stop Solution should be added to wells in the same order and timing as the TMB Substrate solution. Gently tap the plate to ensure thorough mixing. The blue color will change to yellow immediately.
18. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

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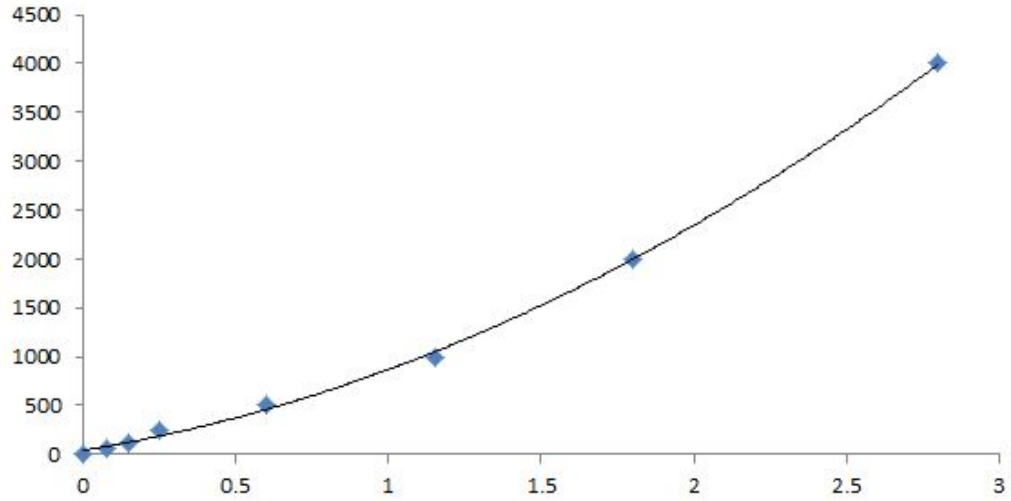
Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average 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 x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. Use of a commercial software program such as CurveExpert is recommended for performing these calculations. 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. The following standard curve is an example only and should not be used to calculate results for tested samples. A new standard curve must be generated for each set of samples tested.



Important Note: During shipment small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

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Technical Support

All requests for technical support should be submitted to Technical.Support@LSBio.com.

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