ab131556 – Human TLR2 ELISA Kit (CD282)

Instructions for Use

For quantitative detection of Human TLR2 (CD282) in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

This product is for research use only and is not intended for diagnostic use.

Version 3a Last Updated 25 January 2024

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1. BACKGROUND

Abcam's Human TLR2 (CD282) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Human soluble TLR2 in cell culture supernatants, serum and plasma (heparin, EDTA).

A TLR2 specific mouse monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat, specific for TLR2 is then added followed by washing with 1X Wash Buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with 1X Wash Buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human TLR2 amount of sample captured in plate.

Toll-like receptor 2 also known as TLR-2 is a protein that in Humans is encoded by the TLR2 gene. TLR2 has also been designated as CD282 (cluster of differentiation 282). TLR-2 plays a role in the immune system. TLR-2 is a membrane protein, a receptor, which is expressed on the surface of certain cells and recognizes foreign substances and passes on appropriate signals to the cells of the immune system. TLR2 expresses as 4- and 4.4-kb mRNAs in heart, brain, and muscle.

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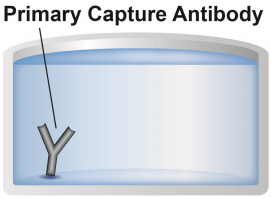
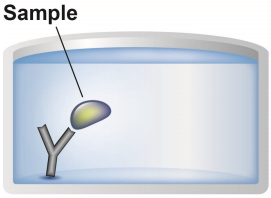
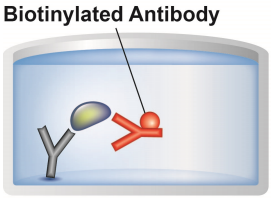
2. ASSAY SUMMARY



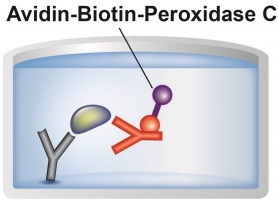
Prepare all reagents, samples and standards as instructed.



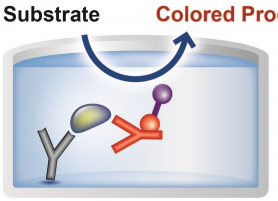
Add standard or sample to each well used. Incubate at room temperature.



Add prepared biotin antibody to each well. Incubate at room temperature.



Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.



Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt. Avoid multiple freeze-thaw cycles.

Refer to list of materials supplied for storage conditions of individual components.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Anti-Human TLR2 antibody Microplate (12 x 8 wells)	96 Wells	-20ºC
Lyophilized recombinant mouse Human TLR2 standard	2 x 10 ng	-20ºC
Biotinylated anti-Human TLR2 antibody	100 µL	-20ºC
Avidin-Biotin-Peroxidase Complex (ABC)	100 µL	-20ºC
Sample Diluent Buffer	30 mL	-20ºC
Antibody Diluent Buffer	12 mL	-20ºC
ABC Diluent Buffer	12 mL	-20ºC
TMB Color Developing Agent	10 mL	-20ºC
TMB Stop Solution	10 mL	-20ºC
Plate Seal	4 units	-20ºC
Wash Buffer (25X)	20 mL	-20ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 **1X Biotinylated anti-Human TLR2 antibody**

Biotinylated anti-Human TLR2 antibody must be diluted 1:100 with the Antibody Diluent Buffer and mixed thoroughly (i.e. add 1 µL Biotinylated anti-Human TLR2 antibody to 99 µL Antibody Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.2 **1X Avidin-Biotin-Peroxidase Complex**

Before use, briefly centrifuge the tubes in case any of the contents are trapped in the lid or sticking to the tube walls. Avidin-Biotin-Peroxidase Complex (ABC) must be diluted 1:100 with ABC Diluent Buffer and mixed thoroughly (i.e. add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume required should be; 100 µL/well multiplied by the total number of wells (allow 100 µL - 200 µL extra for pipetting error).

9.3 **Wash Buffer (25X)**

Prepare 500 mL of working 1X Wash Buffer by diluting 20 ml of the supplied Wash Buffer (25X) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Reconstitution of the Human TLR2 standard should be prepared no more than 2 hours prior to the experiment. Two tubes of TLR2 standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

10.1 Prepare a 10 ng/mL **Standard #1** by reconstituting the TLR2 standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This 10 ng/mL **Standard #1** should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

10.2 Label seven tubes with #2 - 8.

10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

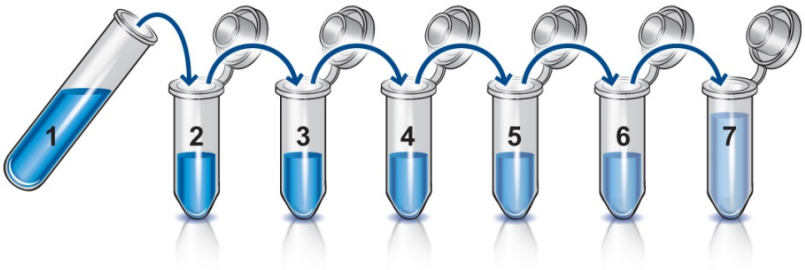
10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #5 through #7.

10.8 **Standard #8** contains no protein and is the Blank control.

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.1				10,000
2	Standard #1	300	300	10,000	5,000
3	Standard #2	300	300	5,000	2,500
4	Standard #3	300	300	2,500	1,250
5	Standard #4	300	300	1,250	625
6	Standard #5	300	300	625	312
7	Standard #6	300	300	312	156
8	None	-	300	-	-



11. SAMPLE COLLECTION AND STORAGE

Store samples to be assayed within 24 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

11.1 Cell Culture Supernatants

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Cell Lysates

After sufficient splitting, there should be no obvious cell sediment. Centrifuge cell lysates at approximately 10,000 x *g* for 5 minutes. Collect the cell lysate supernatants to go ahead.

11.3 Serum

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x *g* for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

11.4 Plasma

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 30 minutes at 1,000 x *g* within 15 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

12. SAMPLE PREPARATION

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the diluent buffer before assaying.

- High target protein concentration (100 – 1,000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL Sample Diluent Buffer
- Medium target protein concentration (10 - 100 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL Sample Diluent Buffer
- Low target protein concentration (156 – 10,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL Sample Diluent Buffer
- Very Low target protein concentration (≤ 156 pg/mL). No dilution necessary, or the working dilution is 1:2.

13. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

14. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

14.1 Prepare all reagents, working standards, and samples as directed in the previous sections

14.2 Add 100 µL of prepared standards and diluted samples to appropriate wells.

14.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.

14.4 Remove the cover, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

14.5 Add 100 µL of 1X Biotinylated anti-Human TLR2 antibody into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 60 minutes.

14.6 Wash the plate three times with 300 µL 1X Wash Buffer, and each time let the wash buffer stay in the wells for one minute. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.

Note: For automated washing, aspirate all wells and wash THREE times with 1X Wash Buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

14.7 Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal the plate with a new plate seal and incubate the plate at 37°C for 30 minutes.

14.8 Wash plate five times with 1X Wash Buffer, and each time let wash buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material. (See Step 14.6 for plate washing method).

14.9 Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 15 - 25 minutes

Note: The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated Human TLR2 standard solutions; the other wells show no obvious color.

14.10 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.

14.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

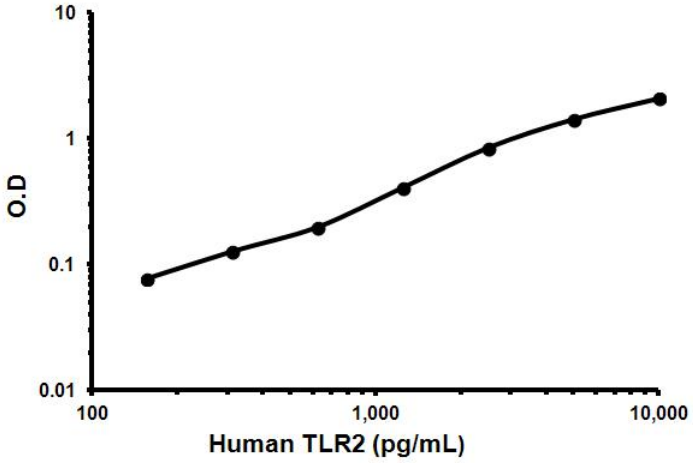
15. CALCULATIONS

For calculation, the relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human TLR2 concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, make sure to account for this in your calculations.

16. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0.0	0.016
156	0.078
312	0.128
625	0.200
1,250	0.412
2,500	0.853
5,000	1.434
10,000	2.097

17. TYPICAL SAMPLE VALUES

RANGE – 156 - 10,000 pg/mL

SENSITIVITY – < 10 pg/mL

18. ASSAY SPECIFICITY

This kit detects both endogenous and recombinant Human TLR2.

No detectable cross-reactivity with other relevant proteins.

19. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

20. NOTES

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)