



Human CD32B ELISA Kit

(Sandwich ELISA)

User Manual

Catalog No. LS-F2970

It is important that you read this entire manual
carefully before starting your experiment.

This kit is for Research Use Only and not for Diagnostic Use.
This kit is not approved for use in humans or for clinical diagnosis.

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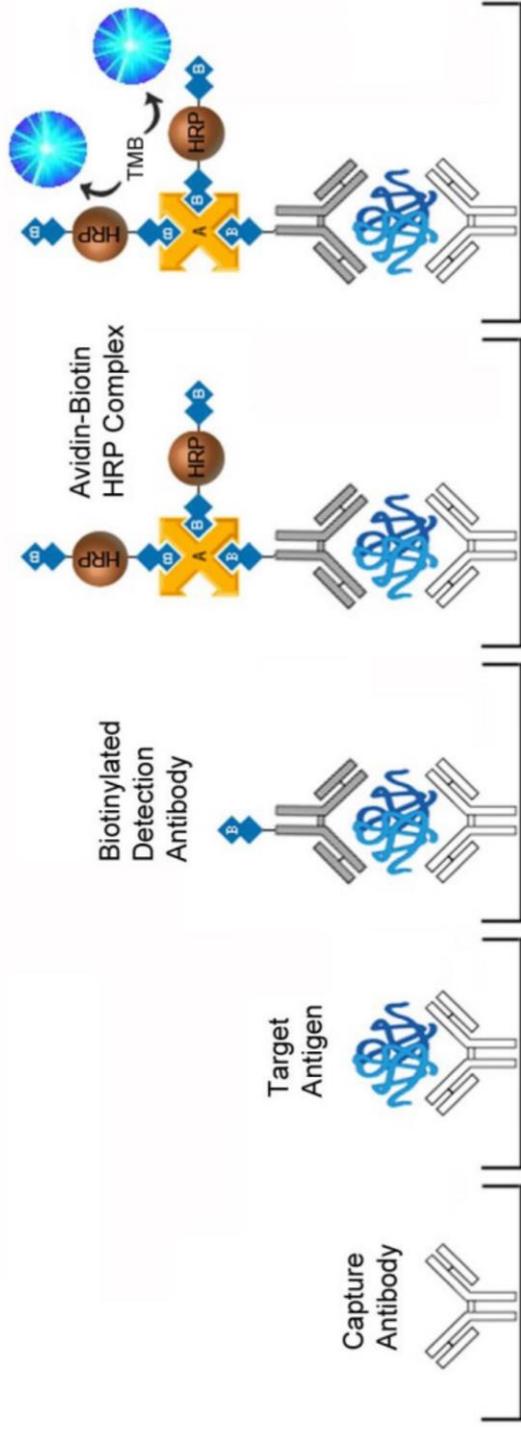
ASSAY SPECIFICATIONS

| | |
|-------------------------|--|
| Target: | CD32B |
| Synonyms: | CD32B, FCGR2B, Fc fragment of IgG, low affinity IIb, receptor (CD32), CD32, CD32 antigen, CDw32, FCG2, FcRII-b, IgG Fc receptor II-b, Fc gamma RIIb, Fc-gamma RII-b, FCGR2, Fc-gamma-RIIb, IGFR2 |
| Specificity: | This kit is for the detection of natural and recombinant human CD32. No significant cross-reactivity or interference between CD32 and analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs. |
| Sample Types: | This kit is recommended for use with Human Cell Culture Supernatants, Plasma, and Serum (Plasma collected using heparin or EDTA). Use with other sample types is not supported. |
| Detection: | Colorimetric - 450nm (TMB) |
| Measurement: | Quantitative |
| Detection Range: | 46.9–3000 pg/ml |
| Sensitivity: | Typically less than 1 pg/ml |
| Performance: | Intra-Assay CV (<4.5%); Inter-Assay CV (<5.8%) |
| Limitations: | This kit is for Research Use Only and is not intended for diagnostic use. This kit is not approved for use in humans or for clinical diagnosis. |

ASSAY PRINCIPLE

This assay is based on the sandwich ELISA principle. Each well of the supplied microtiter plate has been pre-coated with a target specific capture antibody. Standards or samples are 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 Biotin-Peroxidase Complex (ABC) is then added which binds to the biotin and amplifies the signal. Unbound ABC is washed away. A TMB substrate is then added which reacts with the peroxidase 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 $450 \text{ nm} \pm 2 \text{ nm}$. The OD of an unknown sample can then be compared to an OD standard curve generated using known antigen concentrations in order to determine its antigen concentration.

ASSAY PRINCIPLE IMAGE



KIT COMPONENTS AND STORAGE

| Component | Quantity |
|--|----------------------|
| Coated 96-well Strip Plate | 1 |
| Standard (Lyophilized) | 2 vials |
| Sample Diluent | 1 vial x 30 ml |
| Biotinylated Detection Antibody (100x) | 1 vial x 130 μ l |
| ABC Complex (100x) | 1 vial x 130 μ l |
| Detection Antibody Diluent | 1 vial x 12ml |
| ABC Complex Diluent | 1 vial x 12ml |
| TMB Substrate | 1 vial x 10ml |
| Stop Solution | 1 vial x 10 ml |
| Adhesive Plate Sealers | 4 |
| Instruction Manual | 1 |

KIT STORAGE

The unopened kit can be stored at 2-8°C through the expiration date. Once opened, all kit components can be stored at 2-8°C of up to 1 month. For long term storage the Standard, Biotin-antibody, and HRP-avidin should be stored at -20°C. TMB Substrate should always be stored at 4°C. Unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air.

OTHER REQUIRED SUPPLIES

- ☐ Microplate reader with 450nm wavelength filter
- ☐ High-precision pipette and sterile pipette tips
- ☐ Eppendorf tubes
- ☐ 37°C incubator
- ☐ Deionized or distilled water
- ☐ Absorbent paper
- ☐ Plate Sealers
- ☐ Wash Buffer (see Reagent Preparation)
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SAMPLE COLLECTION NOTES

1. LSBio recommends that samples are used immediately upon preparation. Alternatively, samples stored at 2-8°C should be used within 5 days. For long-term storage sample aliquots should be prepared and stored at -20°C if used within 1 month, or -80°C if used within 6 months. Long term storage can result in protein degradation and denaturation, which may result in inaccurate results.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. LSBio is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer

REAGENT PREPARATION

Bring all reagents to room temperature (18-25°C) before use. ABC Complex and TMB solutions should be warmed to 37°C for 30 minutes prior to use in the assay.

1x Detection Antibody: Centrifuge the vial before opening. Prepare 1 ml of working Detection Antibody solution by diluting 10 µl of Detection Antibody concentration (100x) with 990 µl of Detection Antibody Diluent. Use within 2 hours.

1x ABC Complex: Centrifuge the vial before opening. Prepare 1 ml of working ABC Complex solution by diluting 10 µl of ABC Complex concentration (100x) with 990 µl of ABC Complex Diluent. Use within 2 hours.

Wash Buffer: Either 0.01M PBS or 0.01M TBS can be used as Wash Buffer.

Preparation of 0.01M TBS: Add 1.2 g Tris, 8.5 g NaCl; 450 µl of purified acetic acid or 700 µl of concentrated hydrochloric acid to 1000 ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01M PBS: Add 8.5 g sodium chloride, 1.4 g Na₂HPO₄ and 0.2 g NaH₂PO₄ to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

SAMPLEPREPARATION

Please predict the concentration of your samples before assaying. The resulting optical density (OD) values of your sample must fall within the OD readings of the standard curve in order for the calculated antigen concentration to be accurate. A dilution series of each sample may be necessary. Running duplicate wells for each sample is recommended.

Always dilute samples in the same buffer as the Standard used to generate the Standard Curve.

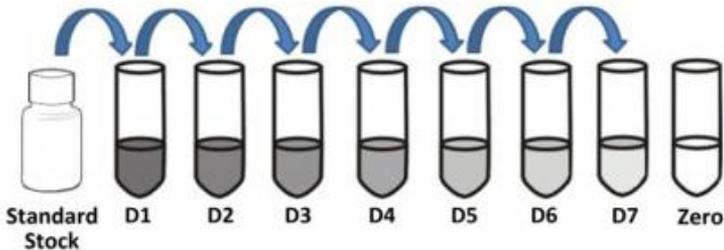
STANDARD PREPARATION

The following are instructions for the preparation of a Standard dilution series which will be used to generate the standard curve. The standard curve is then used to determine the concentration of target antigen in unknown samples (see the **Calculation of Results** section). The following will prepare sufficient volumes to run the Standard dilution series in duplicate. Reconstituted Standard and prepared standard dilutions should be used immediately and not stored for future use.

Standard Stock Solution (10,000 pg/ml): Reconstitute 1 tube of lyophilized Standard with 1.0 ml of Sample Diluent. Incubate at room temperature for 10 minutes with gentle agitation (avoid foaming).

- D1** (3000 pg/ml): Pipette 300 μ l of Stock Standard into 700 μ l of Sample Diluent
- D2** (1500 pg/ml): Pipette 250 μ l of D1 into 250 μ l of Sample Diluent
- D3** (750 pg/ml): Pipette 250 μ l of D2 into 250 μ l of Sample Diluent
- D4** (375 pg/ml): Pipette 250 μ l of D3 into 250 μ l of Sample Diluent
- D5** (187.5 pg/ml): Pipette 250 μ l of D4 into 250 μ l of Sample Diluent
- D6** (93.75 pg/ml): Pipette 250 μ l of D5 into 250 μ l of Sample Diluent
- D7** (46.88 pg/ml): Pipette 250 μ l of D6 into 250 μ l of Sample Diluent

Zero Standard (0 pg/ml): Use Sample Diluent alone



REAGENT PREPARATION NOTES

1. It is highly recommended that standard curves and samples are run in duplicate within each experiment.
2. Once resuspended, standards should be used immediately, and used only once. Long-term storage of reconstituted standards is NOT recommended.
3. All solutions prepared from concentrates are intended for one-time use. Do not reuse solutions.
 - Do not prepare Standard dilutions directly in wells.
4. Prepared Reagents may adhere to the tube wall or cap during transport; centrifuge tubes briefly before opening.
 - All solutions should be gently mixed prior to use.
5. Reconstitute stock reagents in strict accordance with the instructions provided.
 - To minimize imprecision caused by pipetting, ensure that pipettes are calibrated. Pipetting volumes of less than 10 μ L is not recommended.
6. Substrate Solution is easily contaminated; sterility precautions should be taken. Substrate Solution should also be protected from light.
10. Do not substitute reagents from one kit lot to another. Use only those reagents supplied within this kit.
11. Due to the antigen specificity of the antibodies used in this assay, native or recombinant proteins from other manufacturers may not be detected by this kit.

ASSAYPROCEDURE

Bring all reagents and samples to room temperature without additional heating and mix thoroughly by gently swirling before pipetting (avoid foaming). Prepare all reagents, working standards, and samples as directed in the previous sections.

- 1 Add 100 μ l of **Standard, Blank, or Sample** per well, cover with a plate sealer, and incubate for 90 minutes at 37°C.
 - Aspirate the liquid of each well, don't wash.
- 2 Add 100 μ l of **1x Detection Antibody** working solution to each well, cover with a plate sealer, and gently agitate to ensure thorough mixing. Incubate for 1 hour at 37°C.
 -
- 3 Aspirate the liquid from each well and wash 3 times. Wash by adding approximately 350 μ l of Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Allow each wash to sit for 1-2 minutes before completely aspirating. After the last wash, aspirating remove any remaining Wash Buffer then
4. invert the plate and tap against clean absorbent paper.

Add 100 μ l of **1xABC Complex** working solution to each well, cover with a new plate sealer, and incubate for 30 minutes at 37°C.

Aspirate the liquid from each well and wash 5 times as outlined in step 4.

- 5 Add 90 μ l of **TMBSubstrate** to each well, cover with a new plate sealer, and incubate for ~15-30 minutes at 37°C while protecting from light. Incubation time is estimated and wells should be monitored periodically until the optimal color development has been achieved. Wells with high concentrations of Standard will turn blue.
 -
- 6 Add 100 μ l of **Stop Solution** to each well. The blue color will change to yellow immediately. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. The Stop Solution should be added to wells in the same order and timing as was the substrate solution.
- 7

Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm.

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ASSAYPROCEDURENOTES

1. **ELISA Plate:** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be placed in a sealed bag containing desiccant and stored at 4°C.
2. **Solutions:** In the event that Detection Reagent A working solution appears cloudy, warm to room temperature and mix gently until solution appears uniform. To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. **Applying Solutions:** All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
4. **Assay Timing:** The interval between adding sample to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
5. **Incubation:** To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
6. **Washing:** Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
7. **Controlling Substrate Reaction Time:** After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. Excessively strong color will result in inaccurate absorbance reading.

8. **Reading:** The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
9. **Reaction Time Control:** Control reaction time should be strictly followed as outlined.
10. **Stop Solution:** The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
11. **Mixing:** During incubation times, the use of a micro-oscillator at low frequency is recommended. Sufficient and gentle mixing is particularly important in producing reliable results.
12. Kits from different batches may be a little different in detection range, sensitivity, and color developing time. Please perform the experiment exactly according to the supplied instructions.
13. Due to inter- and intra-assay variability, it is recommended that appropriate carry-over controls be included between assays.
14. Prior to running valuable samples, LSBio recommends that the user run a preliminary experiment using the supplied controls in order to validate the assay.
15. To minimize external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.
16. The kit should not be used beyond the expiration date on the kit label.

ASSAYPROCEDURESUMMARY

Prepare all reagents, samples and standards.

Add 100 μ l of **Standard** or **Sample** to each well and incubate for 90 minutes at 37°C.

Aspirate and add 100 μ l of **Detection Antibody** and incubate of 1 hour at 37°C.

Aspirate and wash 3 times.

Add 100 μ l of **ABC Complex** and incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add 90 μ l of **TMBSubstrate** and incubate for ~15-30minutes at 37°C.

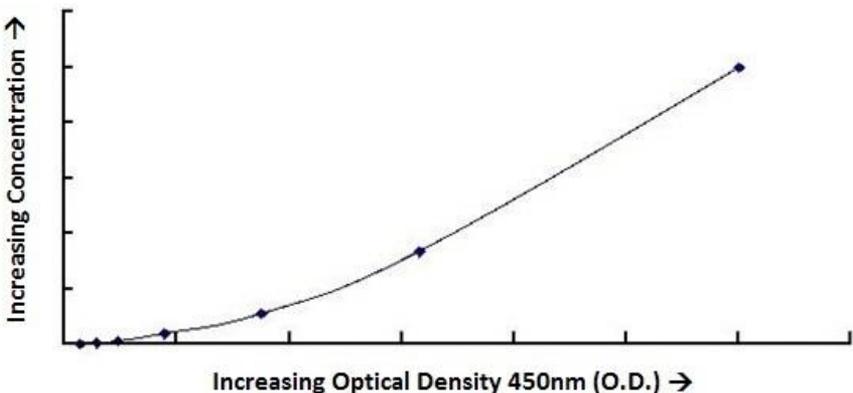
Add 100 μ l of **Stop Solution**.

Read immediately at 450nm.

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.

Typical Data: 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.



TROUBLESHOOTING GUIDE

| Problem | Possible Cause | Solution |
|--------------------------|-----------------------------------|---|
| Poor standard curve | Inaccurate pipetting | Check pipettes |
| | Improper standard dilution | Briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix. |
| | Wells not completely aspirated | Completely aspirate wells between steps. |
| Low signal | Too brief incubation times | Ensure sufficient incubation time. |
| | Incorrect assay temperature | Use recommended incubation temperature. Bring substrate to room temperature before use. |
| | Inadequate reagent volumes | Check pipettes and ensure correct preparation. |
| | Improper dilution | |
| Deep color but low value | Plate reader settings not optimal | Verify the wavelength and filter setting in the plate reader. |
| | | Open the Plate Reader ahead to pre-heat. |

Troubleshooting Guide (continued)

| Problem | Possible Cause | Solution |
|-----------------|------------------------------------|--|
| Large CV | Inaccurate pipetting | Check pipettes |
| High background | Concentration of detector too high | Use recommended dilution factor. |
| | Plate is insufficiently washed | Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. |
| | Contaminated wash buffer | Make fresh wash buffer. |
| Low sensitivity | Improper storage of the ELISA kit | All the reagents should be stored according to the instructions. |
| | Stop solution not added | Stop solution should be added to each well before measurement. |

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.

Warning: This reagent may contain sodium azide and sulfuric acid. The chemical, physical, and toxicological properties of these materials have not been thoroughly investigated. Standard Laboratory Practices should be followed. Avoid skin and eye contact, inhalation, and ingestion. Sodium azide forms hydrazoic acid under acidic conditions and may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent accumulation.

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