ab212086
Human CD44 ELISA Set

Instructions for use:

For the quantitative measurement of CD44 in Human serum, plasma, buffered solutions and cell culture media.

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

Version 7 Last Updated 26 July 2016

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1. BACKGROUND

Abcam's CD44 Human *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) Set (ab212086) is designed for the quantitative measurement of soluble CD44 in Human serum, plasma, buffered solutions and cell culture medium. The ELISA set contains 100 µg of unlabelled, mouse monoclonal capture antibody and 50 µg of unlabelled mouse monoclonal detector antibody. Lyophilized Human CD44 protein purified from Human serum is included for quantification. The exact concentration of the protein standard can be found on the vials' label. Detection requires a secondary antibody labelled with biotin.

The protocol booklet contains guidelines on how to use the ELISA Set for a sandwich ELISA with a secondary antibody for detection.

This kit will recognize both endogenous and recombinant Human CD44.

Optimization of the ELISA Set reagents to sample type, immunoassay format or instrumentation may be required. Guidelines for use of this ELISA Set in a standard 96-well microplate sandwich ELISA using HRP/TMB system of colorimetric detection is described in this assay procedure for the purposes of quantification.

Additional protocol information and tip on the use of the Human CD44 ELISA Set for sandwich ELISA can be found on our website:

http://www.abcam.com/kits/how-to-use-matched-antibody-pair-kits-for-sandwich-elisa

For additional information on the performance of the antibody pair used in this ELISA Set, please see our equivalent CD44 Human ELISA Set (ab45912) which uses the same antibody pair.

2. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- The Stop Solution suggested for use with this ELISA Set is a concentrated acid solution and should be used with caution and adequate personal protective equipment (PPE).
- The TMB substrate suggested for use with this Set may cause skin, eye, and respiratory irritation. Wear PPE. Wash hands thoroughly after handling.
- Please review the SDS on the Abcam website prior to use.

3. STORAGE AND STABILITY

Store ELISA Set at -20ºC immediately upon receipt. Do not use past the expiration date.

4. LIMITATIONS

The supplied ELISA Set is intended for research use only. Not for use in diagnostic procedures.

This ELISA Set contains sufficient materials to perform 10 x 96-well sandwich ELISAs provided the following conditions are met:

- The reagents are prepared as described in the booklet.
- The assay is run as described in the Assay Procedure.
- The recommended reagents and solutions are used.
- Exact conditions may vary from assay to assay, a standard curve should be generated for every assay performed.

Additional considerations:

- Bacterial or fungal contamination of either samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing fresh 1X Wash Buffer. Do not allow wells to sit uncovered or dry for extended periods.
- Do not extrapolate the standard curve beyond the maximum standard curve point. The dose-response is non-linear in this region and good accuracy is difficult to obtain. Concentrated samples above the maximum standard concentration must be diluted with sample buffer to produce an OD value within the range of the standard curve.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Human CD44 Capture Antibody	100 µg	4ºC	4ºC
Human CD44 Detector Antibody	50 µg	4ºC	4ºC
Human CD44 Standard Protein	139 ng	4ºC	-80ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the ELISA Set, but will be required to successfully perform this assay:

- Nunc MaxiSorb 96-well plates (ab210903).
- 10X Phosphate Buffered Saline (PBS) (ab128983): 1% KCl, 1% K_2HPHO_4, 1% NaH_2PO_4, 1% NaCl, pH 7.2-7.5, 0.2 µm filtered.
- Coating buffer (ab210899): 29.4% $NaHCO_3$, 15.9% Na_2CO_3, pH 9.6, 0.2 µm filtered.
- Blocking buffer (ab210904): 1% BSA*, 0.05% Tween® 20, in 1X PBS, pH 7.2-7.4, 0.2 µm filtered.

  *We recommend ≥96% purity BSA as less pure BSA can increase background
- Wash buffer (ab206977): 0.05% Tween® 20 in 1X PBS, pH 7.2-7.4.
- 1X TMB substrate (ab210902).
- 1X Stop solution (ab210900): 4.9% orthophosphoric acid.
- Biotinylated secondary antibody
- 500X HRP conjugated streptavidin: 50 µg/mL HRP conjugated streptavidin (ab210901).
- Microcentrifuge tubes for dilution of standards
- Double distilled water (ddH_2O).
- Optional: Protease Inhibitor Cocktail (ab65621).
- Optional: BCA Protein Quantification Kit (ab102536).

Required Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Multi-channel and single-channel pipettes.

Recommended Equipment

- Plate shaker or rocker.
- Plate washer.

7. TECHNICAL HINTS

- Samples which measure higher absorbance values than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Change tips in between pipetting of samples, standards and reagent additions to avoid cross contamination.
- Ensure plates are properly sealed or covered during incubation steps to prevent the plate from drying out and from presenting edge effects.
- Completely remove all solutions and buffers from the plate during wash steps to minimize background.
- All samples should be mixed thoroughly but gently.
- Minimize freeze/thaw cycles of samples, standards and antibodies.

Please see http://www.abcam.com/protocols/elisa-troubleshooting-tips for more information on improving assay performance.

Note: Before starting the reagent preparation and the assay procedure, please ensure that the recommended additional materials are prepared or available and ready to use.

8. REAGENT PREPARATION

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Allow all reagents to sit for a minimum of 15 minutes with gentle shaking after the initial reconstitution.
- Dilute reagents to 1x working concentrations.
- Reagent dilutions should be prepared and used immediately.
- Prepare only as much reagents as is needed on the day of the experiment.

8.1 Capture Antibody

Dilute Capture Antibody from 1 mg/mL to the suggested working concentration of 2 µg/mL in the Coating Buffer. Add 50 µL per well.

e.g. For one plate:

10 µL of 1 mg/mL Capture Antibody

4,990 µL of Coating Buffer

Note: For best results in your application, optimization of the concentration of capture antibody in the coating buffer may be required.

8.2 Detector Antibody

Dilute the Detector Antibody from stock concentration of 0.25 mg/mL to the suggested working concertation of 0.5 µg/mL in Blocking Buffer or other appropriate diluent. Add 50 µL per well.

e.g. For one plate:

10 µL Detector Antibody

4,990 µL of Blocking Buffer

Note: For best results in your application, optimization of the concentration of detector antibody in the blocking buffer may be required.

8.3 PLATE PREPARATION

8.3.1 Add 50 µL of 2 µg/mL Capture antibody to each well of a 96 well microplate and seal the plate with a plate seal. Incubate the plate either overnight at 4°C or for 2 hours at room temperature on a plate rocker or shaker.

8.3.2 Wash plate three times with 350 µL of the recommended Wash Buffer. Wash by aspirating or decanting from wells then dispensing 350 µL Wash Buffer into each well. Complete removal of liquid after the wash step is essential for good performance. Repeat two more times for a total of three washes. After the last wash step, invert the plate and blot it against clean paper towels to remove excess liquid.

8.3.3 Reduce non-specific binding of proteins in the sample or the Detector Antibody by incubating plates with 300 µL of Blocking Buffer to per well. Cover plate with plate seal or plate cover. Incubate plate either overnight at 4°C or for 2 hours at room temperature.

8.3.4 Repeat the wash procedure in step 8.3.2.

8.3.5 Plates can be used immediately or can be air dried, for minimum 12 hours at room temperature. Air dried plates can be sealed and stored at 4°C for up to 2 weeks before use.

9. STANDARD PREPARATION

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use.

The following section describes the preparation of a two-fold diluted standard curve for duplicate measurements (recommended).

9.1 Prepare a 10 ng/mL Standard #1 by reconstituting with the appropriate volume of 1X Standard Diluent Buffer (available in CD44 ELISA kit (ab45912)) or PBS.

9.2 Label tubes #2-6 and add 100 µL of 1X Standard Diluent Buffer or PBS into each tube.

9.3 Prepare Standard #2 by adding 100 µL of Standard #1 to tube #2 and mix thoroughly.

9.4 Prepare Standard #3 by adding 100 µL of Standard #2 to tube #3 and mix thoroughly.

9.5 Using the table below as a guide, prepare further serial dilutions.

9.6 1X Standard Diluent Buffer or PBS (contains no proteins) serves as the zero standard (0 ng/mL).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	-	-	-	10	10
2	100	100	200	10	5
3	100	100	200	5	2.5
4	100	100	200	2.5	1.25
5	100	100	200	1.25	0.62
6	100	100	200	0.62	0.31

10. SAMPLE PREPARATION AND STORAGE

- Preparation of Plasma Samples

  Collect plasma using citrate, EDTA or heparin. Centrifuge samples at 1,000 x g for 30 minutes. Dilute samples 1:40 into 1X Standard Diluent Buffer and assay. Store un-diluted plasma samples at -20ºC or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- Preparation of Serum Samples

  Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 1,000 x g for 10 minutes and collect serum. Dilute samples 1:40 into 1X Standard Diluent Buffer and assay. Store un-diluted serum at -20ºC or below. Avoid repeated freeze-thaw cycles.

- Preparation of Cell culture Supernatants

  Centrifuge cell culture media at 1,000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

11. ASSAY PROCEDURE

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

11.1. Prepare all reagents, working standards, and samples as described in the previous sections.

11.2. Add 50 µL of diluted sample or standard solution to the appropriate wells of plate prepared in step 8.3. Seal the plate and incubate for 2 hours at room temperature on a plate shaker set to 400 rpm.

11.3. Wash plate three times with 350 µL of the recommended Wash Buffer. Wash by aspirating or decanting from wells then dispensing 350 µL Wash Buffer into each well. Complete removal of liquid after the wash step is essential for good performance. Repeat two more times for a total of three washes. After the last wash step, invert the plate and blot it against clean paper towels to remove excess liquid.

11.4. Add 50 µL of the Detector Antibody diluted in Blocking Buffer to each well. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

11.5. Repeat wash step as described in step 10.3.

11.6. Add secondary antibody diluted in Blocking Buffer in accordance with the manufacturer's suggestion for ELISA.

11.7. Repeat wash step as described in step 10.3.

11.8. Add 50 µL of 1X HRP-conjugated Streptavidin solution, diluted in 1X Wash Buffer to each well. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

11.9. Repeat wash step as described in step 10.3.

11.10. Add 100 µL of TMB Substrate to each well and incubate for up to 20 minutes in the dark on a plate shaker set to 400 rpm.

11.11. Add 100 µL of Stop Solution to each well. Place plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm.

11.12. Analyze the data as described below.

12. CALCULATIONS

12.1. Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

12.2. **Create a standard curve** by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.

Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 5 parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

12.3. Determine the concentration of the target protein in the sample by interpolating the blank control subtracted **absorbance values against the standard curve**. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.

12.4. Samples generating absorbance values greater than that of the highest standard should be further diluted and re-analyzed. Similarly, samples which measure at an absorbance values less than that of the lowest standard should be retested in a less dilute form.

13. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed. The linear range of the assay is 10 – 0.31 ng/mL.

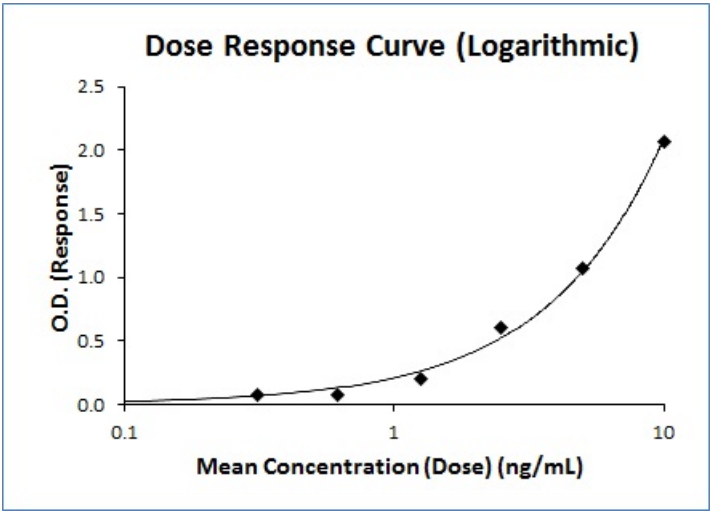

Figure 1 Dose response curve.

14. TROUBLSHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Calibrate pipettes
	Improper standard 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; increase to 2 or 3 hour for standard and sample incubation step
	Inadequate reagent volumes or improper dilution	Calibrate pipettes and ensure correct preparation
	Incubation times with substrate too brief	Ensure sufficient incubation time until blue color develops prior addition of stop solution
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 ELISA Set reagents	Store your reconstituted standards at -80ºC and antibodies at 4ºC. Keep TMB substrate solution protected from light.

15. NOTES

RESOURCES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

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