Human CD48 ELISA Kit

Cat #: orb864382 (manual)

Reactivity	Human			**Range**	62.5–4000pg/ml			
Size	96T/48T			**Sensitivity**	12.5pg/ml			
Validity	6 months			**Storage**	4℃			
Repeatability	the coefficients of variation within and between plates were less than 10%.							
Specificity	it can detect Human CD48 in samples, and has no obvious cross reaction with its analogues.							
Standard Curve (pg/ml)	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**Blank**
	4000.0	2000.0	1000.0	500.0	250.0	125.0	62.5	0
Application	this kit is used for qualitative or quantitative analysis in vitro, only for scientific research, not for clinical diagnosis, and for serum, plasma, tissue homogenate, cell culture supernatant and other biological fluid.							

- **Introduction**: CD48 antigen (Cluster of Differentiation 48), also known as BLAST-1 or SLAMF2, is a protein that in humans is encoded by the CD48 gene. CD48 is a member of the CD2 subfamily of the immunoglobulin superfamily (IgSF) which includes SLAM (signaling lymphocyte activation molecules) proteins, such as CD84, CD150, CD229 and CD244. It is mapped to 1q23.3. CD48 is found on the surface of lymphocytes and other immune cells, dendritic cells and endothelial cells, and participates in activation and differentiation pathways in these cells. The encoded protein does not have a transmembrane domain, however, but is held at the cell surface by a GPI anchor via a C-terminal domain which maybe cleaved to yield a soluble form of the receptor.

Test principle

The ELISA Kit adopts the "sandwich method": the capture antibody is coated on the enzyme plate, and the target protein in the sample and standard sample is captured. The biotinylated detection antibody is combined with the target protein, and the SABC complex is combined with the biotinylated detection antibody to form an immune complex. After the TMB chromogenic solution is added, if there is a target protein in the reaction pore, it will be blue, Add the termination solution to turn yellow, and the free components are washed away during the detection process. The OD value is measured at 450 nm with the microplate reader. The concentration of target protein is proportional to the OD value. The concentration of target protein in the sample is calculated by drawing the standard curve. So as to carry out qualitative or relative quantitative analysis on the test sample.

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Kit components & Storage（An unopened kit can be stored at 4℃）

Item	Specifications（48 T）	Specifications（96 T）
Microplate (Dismountable)	8 wells×6 strips	8 wells×12 strips
Standard S(10×)	1 vial	1 vial
Standard & Sample Diluent Buffer	15ml	15ml
Biotinylated Antibody (100×)	1 vial	1 vial
Biotinylated Antibody Diluent Buffer	6ml	12ml
Avidin-Biotin-Peroxidase Complex（SABC）(100×)	1 vial	1 vial
SABC diluent Buffer	6ml	12ml
TMB Solution（A）	3ml	6ml
TMB Solution（B）	3ml	6ml
Stop Solution	3ml	6ml
Wash Buffer (30×)	30ml	30ml
Sealing paper	2	4
Instruction manual	1	1

※Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Sample Collection and Storage (universal)

- **Serum:** after 10-20 minutes of natural coagulation of blood at room temperature, centrifugation for about 20 minutes (2000-3000 rpm/min). Collect and clear- 20 ° C or -80 ° C cryopreservation.
- **Plasma:** Collect plasma using (EDTA-Na2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect and clear- 20 ° C or -80 ° C cryopreservation. Avoid hemolysis, high cholesterol samples.
- **Urine, hydrothorax and ascites, cerebrospinal fluid and bronchoalveolar lavage fluid:** collected with sterile tube. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefully- 20 ° C or - 80 ° C cryopreservation.

- **Cell culture supernatant:** when detecting secretory components, collect them with sterile tube. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefully. The cell suspension was diluted with PBS, and the cell concentration was about 1million/ml. Through repeated freezing and thawing (2-3 quick freeze-thaw cycles of samples using liquid nitrogen and 37ºC water bath), the cells are destroyed and released. Centrifugation for about 20 minutes (2000-3000 rpm/min). Collect the supernatant carefully (Extract fractions for BCA protein quantification if needed), -20°C or - 80°C cryopreservation.
- **Tissue Homogenates:** After cutting the specimen, weigh it. Adding a certain amount of PBS, according to the ratio of weight (g): volume (mL) = 1:9, add 9 times the volume of homogenization medium PBS. The specimens were homogenized by hand or homogenizer. Centrifugation for about 20 minutes (2000-3000 rpm/min). The homogenate supernatant was collected carefully (Extract fractions for BCA protein quantification if needed), -20 °C or -80 °C cryopreservation.

Precautions for sample collection:

1. The volume of each sample collection is about 60µl × detection index. If the hole is to be drilled, the volume of sample collection is about 60µl × detection index × 2. More holes are repeated and soon.
2. Before collecting samples, it must be clear whether the components to be tested are stable enough to determine the storage temperature of samples. The storage conditions refer to sample storage.
3. when collecting serum samples, attention should be paid to avoid hemolysis. When red blood cells dissolve, substances with peroxidase activity will be released. In the ELISA determination marked with HRP, hemolytic samples may increase non-specific color.
4. In order to ensure the accuracy of urine test results, urine samples must be collected and preserved correctly. The urine container should be clean and dry. It is best to use disposable containers (such as plastic urine cup) to avoid contamination caused by medication and unclean cleaning, which will affect the test results. Urine samples must be fresh, after retention, should be timely detection or preservation, in order to avoid bacterial reproduction. It interferes with the detection because of the precipitation of phosphate in urine after long-term storage at room temperature (especially in summer).
5. After thawing the frozen sample, the protein is locally concentrated and unevenly distributed. It should be fully mixed gently to avoid bubbles. It can be mixed upside down, and do not vibrate strongly on the mixer. Repeated freezing and thawing of samples should be avoided.
6. Turbid or precipitated samples should be centrifuged or filtered before detection.
7. Repeated freezing and thawing will reduce the potency of the protein, so if the samples to be tested need to be preserved for multiple tests, they should be stored in a small amount of ice. Appropriate preservatives can also be added.
8. Aprotinin should be added to hormone samples.

Sample preservation:

1. Storage at 4 ° C: samples tested for1-4 days should be stored at low temperature if the time exceeds.
2. Preservation at - 20 ° C or - 70 ° C: the samples for testing on the day after collection shall be stored at 4 ° C for standby. If the samples need to be collected periodically for special reasons, the samples shall be packed in time and stored at - 20 ° C or - 70 ° C. Avoid repeated freezing and thawing.
3. In general, specimens can be stored for 48 hours at 2-8 ° C and for 1 month at - 20 ° C It can be stored for 6 months at 70 ° C.

Precautions

1. It is recommended to make double hole test for standard and sample, and make standard curve for each test.
2. The washing process is very important. If the washing is not sufficient, the accuracy error and OD value will rise wrongly. The concentrated washing solution taken from the refrigerator may crystallize, which is a normal phenomenon. The washing solution can be prepared after the crystallization is completely dissolved in 37 ℃ water bath.
3. All reagents should be recovered to room temperature when testing, and the remaining strips should be sealed after the strips are unsealed and used up within 1 month after putting them back in the bag.
4. The kit uses hypersensitive TMB solution. If the color is too deep, precipitation will appear, which is a normal phenomenon. It can be mixed well without affecting the resµlt interpretation
5. Please wear lab clothes and latex gloves for protection during the test.
6. Kit components of different batches cannot be mixed (except reaction termination solution).
7. EP tube and suction head used in the test are disposable, and mixed use is strictly prohibited.

Avoid using the reagents from different batches together.

Reagent Preparation and Storage

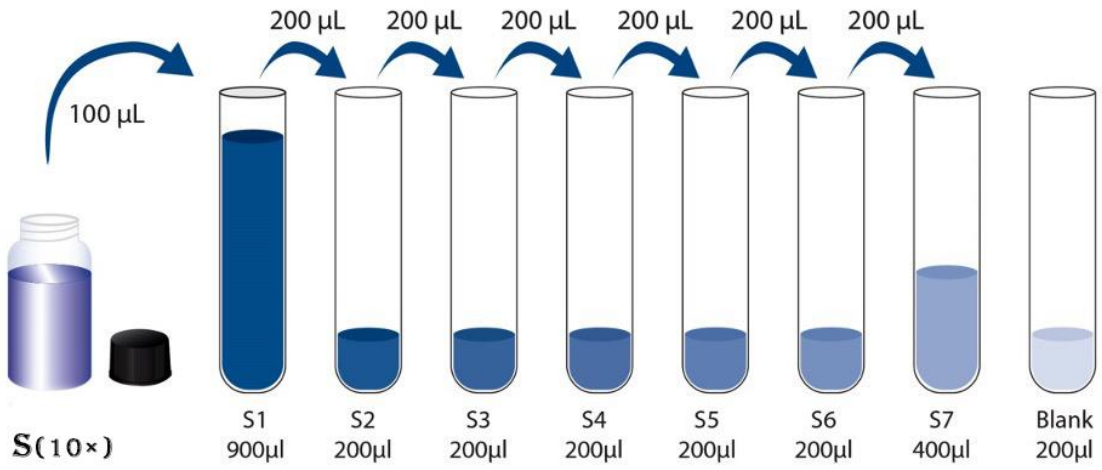
Bring all reagents and samples to room temperature for 20 minutes before use.

1. Wash Buffer:

Dilute with distilled water at 1:30 (for example, 1ml concentrated washing liquid is added with 29ml distilled water).

2. Standards:

Label 8 EP tubes with **S1,S2,S3,S4,S5,S6,S7,Blank** respectively. Add 900µl of standard / sample diluent into the first tube S1, 200µl of standard / sample diluent into the **S2** to **Blank**, add 100µl of standard **S(10×)** solution into the first tube S1, put it on the vortex mixer, mix well, suck out 200µl with the sampler and move it to the **S2**, so as to double dilute, suck out 200µl from the **S7** and discard it, and the eighth tube is the blank control.



Note: It is best to use Standard Solutions within 2 hours.

3．Preparation of Biotinylated Antibody Working Solution:

Prepare it within 1 hour before experiment.

1) Calculate required total volume of the working solution: 0.1ml / well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
2) Dilute the Biotinylated antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1µl Biotinylated antibody into 99µl Antibody Dilution Buffer.)

4．SABC composite working fluid configuration:

20 minutes before use, dilute 100× concentrated ABC compound with ABC compound diluent into 1× working solution, use it on the same day, and discard the rest.

5．Configuration of TMB color developing solution:

10 minutes before use, mix TMB solution A and B 1:1, and keep away from light for standby.

Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

The matrix components in the sample will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Automatic plate washing: the use of automatic plate washing machine should pay attention to the following points:

1. Before washing the plate, check whether the washing bottle and distilled water bottle are sufficient and whether the waste liquid bottle is full.

2. In the process of self-inspection, pay attention to observe whether the lotion perfusion is smooth and whether the drainage is smooth.

3. In the process of plate washing, attention should be paid to observe whether each hole of the reaction hole is full without overflow, whether each hole is fully absorbed, and to ensure that the washing solution is placed in the hole for a long time.

Assay Procedure A

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

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1. **Sample addition:** Blank wells to 50 µl standard/sample diluent Buffer, 50 µl of standard or sample to be tested were added to the other wells, cover the plate, the reaction plate was mixed well and then placed at 37 ℃ for 50 minutes.
2. **Wash:** Remove the cover and discard the plate content, use 1 × Wash the reaction plate with washing solution for 3 times, and add 1 × Lotion 300 µ l. Shake / soak for 1-2 minutes each time, and print dry on the filter paper.
3. **Biotinylated Antibody:** Add 100µl Biotinylated Antibody working solution into above wells (blank wells addition standards/sample diluent buffer). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 50 minutes.
4. **Wash:** The washing steps are the same as above.
5. **Avidin-Biotin-Peroxidase Complex（SABC）:** Add 100µl of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.
6. **Wash:** The washing steps are the same as above.
7. **TMB Solution:** Add 100µl TMB Solution into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
8. **Stop:** Add 50µl stop solution into each well, mix well, and measure the absorbance at 450nm within 30 minutes.**OD**.

   Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank 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 target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation.

   Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Summary

Step1: Add 50µl standard or sample to each well and incubate for 50 minutes at 37°C.wash plates 3 times.

Step2: Add 100µl Biotinylated antibody working solution to each well and incubate for 50 minutes at 37°C.wash plates 3 times.

Step3: Add 100µl SABC Working Solution into each well and incubate for 30 minutes at 37°C.wash plates 3 times.

Step4: Add 100µl TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

Step5: Add 50µl Stop Solution. Read at 450nm immediately and calculation.

Results judgment and calculation:

1. All OD values should be calculated after subtracting the blank hole value. If the blank hole od is lower than 0.1, it can also be calculated directly.
2. take the concentration of standard product as the horizontal coordinate, OD value as the vertical coordinate, draw the standard curve manually or by software, calculate the corresponding content according to the od value of the sample, and multiply it by the dilution multiple

In case of any inconsistency between the Chinese and English instructions, the Chinese instructions shall prevail.

Statement: limited to the existing conditions and scientific level, it is impossible to conduct a comprehensive identification and analysis of all raw materials. This product may have certain quality and technical risks. The final experimental results and the effectiveness of the reagent are closely related to the operator and temperature. Please be sure to prepare sufficient samples for testing.

Problem analysis:

If the experimental effect is not good, please take photos of the color results in time, save the experimental data, keep the used strips and unused reagents, and then contact our technical support to solve the problem for you. You can also refer to the following information:

Poor standard curve

reason	Solution
Incorrect configuration of standard solution	Confirm that the dilution is correct.
The standard has been degraded	Keep and handle the standard in the recommended way.
The scale of the curve is not suitable	Try to draw curves with different scales
Pipette sampling error	Correct use of calibrated pipettes

No signal

reason	Solution
The incubation time was too short	The samples were incubated overnight at 4 ℃, or the experimental protocol of reagents was followed.
The target content is lower than the detection range	Reduce the dilution ratio of the sample or concentrate the sample.
Sample type not applicable	For the sample type that has not been verified, the detection signal may be weakened or the sample type that has not been verified may not be used as a positive control for simultaneous detection.
The epitope was adsorbed by the pore plate and could not be recognized	Direct or indirect ELISA was used to enhance the ability of peptide detection. The peptide was coupled to a large carrier protein and then coated on a microtitration plate.
Test the compatibility of buffer	Ensure that the test buffer is compatible with the target
Insufficient detection reagent	Follow the experimental scheme of reagents, increase the concentration or dosage of the detection reagent.
Incorrect sample preparation	Ensure proper sample preparation / dilution. The sample may not be compatible with the microtitration plate.

Antibody deficiency	Try different antibody concentrations / dilutions.
The incubation temperature is too low	Be sure to incubate at the correct temperature. All reagents (including pore plates) should be at room temperature or the temperature recommended by the experimental scheme of reagents before the experiment.
Incorrect wavelength	Confirm the wavelength and read the board again.
The orifice plate is strongly washed	Check that the pressure of the automatic washing system is correct. If hand washing, gently suck the rinse buffer.
Drying of enzyme standard plate hole	Do not allow the hole to dry after the determination begins. Seal the orifice plate with a sealing film or tape for all incubation steps.
The reaction speed of enzyme is slow	Prepare substrate solution before use. Ensure that the mother liquor is not expired or contaminated. The incubation time was prolonged.
The kit is not well balanced	Balance the reagents at room temperature for at least 20 minutes to ensure that all reagents have been balanced to room temperature.

The coefficient of variation (CV) is large

reason	Solution
Before reading the board	make sure that there is no bubble in the hole.
Uneven / insufficient pore washing	Check whether all pipe orifices of washing machine are unblocked. Wash with recommended method.
Insufficient mixing of reagents	Ensure that all reagents are fully mixed.
Inconsistent fluid transfer	Correct use of calibrated pipettes
edge effect	Make sure that the pore plate and all reagents are at room temperature.
Sample preparation or storage conditions are inconsistent	Ensure that the sample preparation is consistent, and use the optimal sample storage conditions (e.g. minimize repeated freezing and thawing).

High background

reason	Solution
Insufficient hole washing	Washing was carried out according to the experimental scheme.
Washing buffer contamination	Fresh washing buffer was prepared.
Too many test reagents	Ensure that the reagent is properly diluted or the recommended concentration of the test reagent is reduced.
Blocking buffer invalid	Try different sealers and / or add sealers to the washing buffer.
Salt concentration of incubation / washing buffer	Increasing the salt concentration may decrease the nonspecific and / or the off target interaction.
Too long time after adding termination solution before reading board	Read the board immediately after adding the termination solution.
The antibody showed nonspecific binding	Use appropriate blocking buffer, such as BSA or 5-10% normal serum. In case of direct labeled primary antibody, use the same serum as the first antibody species. In case of non-direct labeled primary antibody, use the same serum as the second antibody

	species. Make sure the hole has been pre treated to prevent non-specific adhesion.
High antibody concentration	Try different dilutions to get the best results.
The substrate incubation was carried out under light	The substrate incubation should be carried out in dark or according to the experimental scheme of the reagent.
After the substrate was added, the precipitate was formed in the pore	Increase the dilution ratio of sample or decrease the concentration of substrate.
Foreign body in microplate	Clean the bottom of the orifice plate.
Deterioration of chromogenic solution or expired reagent	Check the validity of the kit and use it within the validity period
Changes of incubation time and temperature	Operate according to the time and temperature recommended in the manual
Reuse of cover, container or gun head	Replace the used cover plate, container or gun head in time

Low sensitivity

reason	Solution
Improper storage of ELISA Kit	Save all reagents as recommended. Please note that the storage conditions of each reagent may be different.
Insufficient targets	Concentrate the sample or reduce the dilution of the sample.
Deactivation of detection reagent	Ensure that the reporter enzyme / fluorescein has the desired activity.
Incorrect setting of microplate reader	In the detection, make sure that the microplate reader is set to the correct absorption wavelength or excitation / emission wavelength.
The method is not sensitive enough	Replace the more sensitive detection system (e.g. from colorimetric detection to chemiluminescence / fluorescence detection). Change to a more sensitive assay (e.g. from direct ELISA to sandwich ELISA). Prolonging incubation time or increasing temperature.
The effect of micro titration plate adsorption target is not good	The target was covalently bonded to the microtitration plate.
Insufficient substrate	Add more substrates.
Incompatible sample type	For the species of samples that have not been verified, the detection signal may be weakened or not. The tested samples were tested simultaneously as positive controls
Interference of buffer or sample components	Confirm if there are any interfering compounds in the reagent. For example, sodium azide in the antibody inhibits HRP enzymes and EDTA used as anticoagulants in plasma inhibits enzyme reactions.
Mix or mix reagents from different kits	Avoid mixing reagents from different kits.
The kit is not well balanced	Balance the reagents at room temperature for at least 20 minutes to ensure that all reagents have been balanced to room temperature.