Cat No. MBS264965

Human A Disintegrin And Metalloprotease 10(ADAM10) ELISA Kit

This product is suitable for in vitro quantitative detection of human serum, plasma or cell culture supernatant and organizations in the natural and recombinant ADAM10 concentration. Detection of other special sample please contact our technical support. The kit is for research use only. Please read the instructions carefully before using and check the kit components.

This kit employs Double Antibody Sandwich Technique. The principle of Double Antibody Sandwich is based on characteristics of the tested antigen with more than two valances which can identify coated antibody and detection antibody at same time. The specific process is as follows:

1. Connect the specific antibodies and solid phase carriers to form immobilized antibodies. Wash out uncombined antibodies and impurities. Seal the rest binding sites with irrelevant proteins.
2. Join under test with immobilized antibodies for contact reaction. After a while, combine antigens in and antibodies on carriers into the antigens complex. Wash out uncombined antibodies and impurities.
3. Add biotin labeling antibodies to combine with the antigens on immune complexes. Wash out the uncombined biotin labeling antibodies thoroughly. The enzyme amount on the carrier is now positively related to the amount of the tested substance in specimens.
4. Add horseradish peroxidase to label the avidins and incorporate them with the biotin labeling antibodies. Wash out the incorporated enzyme markers thoroughly. The enzyme amount on the carrier is now positively related to the amount of the tested substance in specimens.
5. Add substrates for coloring, and compute the concentration of specimens.

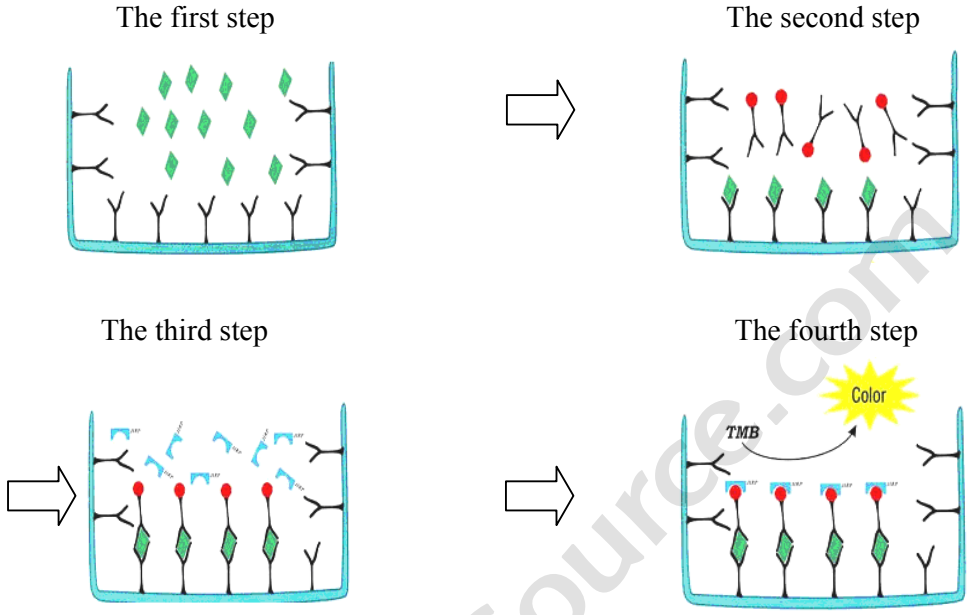
Note: an antibody molecule can be marked on several biotin molecules and a biotin molecule can be connected with a HRP-Avidin to form numbers of horseradish peroxidases combining with antibodies which shows higher sensitivity and amplification effect comparing with traditional direct HRP-Antibodies.

【Detection principle of Human A Disintegrin And Metalloprotease 10(ADAM10) ELISA kit】

This experiment use double-sandwich elisa technique and the ELISA Kit provided is typical. The pre-coated antibody is human ADAM10 monoclonal antibody and the

detecting antibody is polyclonal antibody with biotin labeled. Samples and biotin labeling antibody are added into ELISA plate wells and washed out with PBS or TBS. Then Avidin-peroxidase conjugates are added to ELISA wells in order; Use TMB substrate for coloring after reactant thoroughly washed out by PBS or TBS. TMB turns into blue in peroxidase catalytic and finally turns into yellow under the action of acid. The color depth and the testing factors in samples are positively correlated.

Schematic diagram of the human ADAM10 ELISA kits



【Kit composition】

name	96 Tests	48 Tests	Storage
1. antibody precoated plate	8×12	8×6	4/-20℃
2. Human ADAM10 Standards	2 vial	1 vial	4/-20℃
3. Biotinylated antibody(1:100)	1 vial	1 vial	4/-20℃
4. Enzyme conjugate(1:100)	1vial	1 vial	4/-20℃
5. Enzyme diluent	1vial	1vial	4/-20℃
6. antibody diluent	1vial	1 vial	4/-20℃
7. Standard diluent	1vial	1 vial	4/-20℃
8. Sample diluent	1vial	1 vial	4/-20℃
9. Washing buffer (1:25)	1 vial	1 vial	4/-20℃
10. Colour Reagent A	1 vial	1 vial	4/-20℃
11. Colour Reagent B	1 vial	1 vial	4/-20℃
12. Colour Reagent C	1 vial	1 vial	4/-20℃
13. Instruction	1 set	1 set	RT

Note：

RT：Room temperature

Standard：Frozen dried

Colour Reagent A: Avoid light

【Necessary for testing their own test facilities and equipment】

1. Microplate reader (450nm detection wavelength filter, 570nm or 630nm correction wavelength filters).
2. Washer (adjustable amount of liquid injection to ensure that each well 350μl lotion without overflow).
3. Clean benches, biological safety cabinets, fume hoods.
4. High-precision single-channel dispenser (range 0.5-10μl-20μl, 20-200μl, 200-1000μl).
5. High-precision multi-channel plus liquid (8 or 12, the range of 50-300μl of).
6. 37℃ incubator.
7. Low temperature centrifuge.
8. Refrigerators (4℃, -20℃, -86℃).
9. Analytical balance.
10. Scissors, tweezers, pliers, and so on.
11. Swirl mixing device, low-frequency oscillator, and so on.

【Necessary for testing their own testing supplies and reagents】

1. Centrifuge tube (capacity of 1.5ml, 5ml, and so on).
2. Disposable tip (range of 0.5-10μl-20μl, 20-200μl, 200-1000μl).
3. Pure water or distilled water.
4. Coordinate paper.
5. Absorbent paper.
6. EDTA, sodium citrate, heparin

【Sample collection Note】

1. The tube for blood collection should be free of pyrogen and endotoxin
2. Hemolysis and hyperlipidemia specimens can not be used to extracted serum and plasma.
3. The samples should appear clear and transparent. And all the suspension should be removed through centrifugation.
4. If collected samples are not timely detected, they should be divided according to single usage amount and frozen reserved in refrigerator at -20-80℃, avoiding the repeated freeze-thaw.
5. According to the actual situation of the samples, make proper multiple dilutions (Pre-experiment is strongly recommended in order to confirm the dilution ratio)
6. Collect specimens and try to gain double dosage to avoid specimens shortage for repeated assays in case that failure in one-assay delays experimental process.
7. Do protective measures when collecting specimens (e.g. wearing gloves, respirator, respirator, etc.), aware of the potential risk in all specimens.
8. Specimen processing should be inside the biological safety cabinet. Ensure proper use of the biological safety cabinet.

【Measures for the samples】

1. Serum: Put the collected whole blood in refrigerator at 4℃ for the night. Then

centrifuge it for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20□(for 1-3 months) or -80□ (for 1-3 months) for storage.

2. Plasma: Take EDTA, sodium citrate and heparin as anticoagulant. Add the plasma and mix them well. Centrifuge mixture for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20□(for 1-3 months) or -80□ (for 1-3 months) for storage.
3. Tissue homogenate: Take tissue slices and wash them out in 0.01MPBS; Add tissue protein extraction reagent according to proportion of 1G: 5-10ml and mix them in ice water. After being blended, mixture shall be centrifuged for 10min at 5000-10000rpm.Take supernatant tested immediately or put them at -20□(for 1-3 months) or -80□ (for 1-3 months) for storage.
4. Cell culture: Take centrifugation for 10min at 1000-3000rpm.Take supernatant tested immediately or put samples at -20□(for 1-3 months) or -80□ (for 1-3 months) for storage.
5. For urine, ascites, cerebrospinal fluid, etc: ake centrifugation for 10min at 1000-3000rpm. Take supernatant tested immediately or put samples at -20□(for 1-3 months) or -80□ (for 1-3 months) for storage.

Note: The general principles of the sample dilution

The user should refer to the references to know the probable content of the samples before decide to dilute the samples,and the diluted content of the sample must be in the best detection range of the given ELISA Kits. The dilution of the sample should be recorded in detail.

【Note】

1. The kit should be kept at 2-8℃ before being used. Except the redissolved standard samples, other Ingredients must not be frozen.
2. For the concentrated biotinylated Human ADAM10 antibody, the concentrated enzyme-conjugates have small size. Bumping or potential inversion of the tubes during transportation may cause the liquid sticking to wall or cap. Thus, the tubes should be shaken manually or centrifuged for 1 min at 1000rpm to shake off the adherent liquid down to the tube bottom.
3. Concentrated washing buffer may crystallize a little. Use water bath to help the dissolution during diluting process. The crystals must be totally dissolved when preparing washing buffer.
4. During testing process, the human ADAM10 lyophilized standard sample shall be single-use and must not be divided. The sample will quickly inactivate after being dissolved because of its lower concentration.
5. Operation should be strictly in accordance with the instructions. Mixed usage of components with different batch number in this reagent is not allowed
6. Ensure the reagent well mixed by the spiral hybrid instrument. For the reagent in the microplate, adequate mixing is particularly important to test result. So it's better to employ the micro-oscillator (at the lowest frequency). If there is not micro-oscillator, shake the microplate manually for 1 min, slightly as like a circular movement to make sure reaction liquid in microplate well mixed

7. ELISA for experiment should be strictly operated according to manual standard and fully preheated beforehand.
8. During enzyme immunoassay, there should be multiple pores when testing Human ADAM10 standard samples
9. Put the unused microplates into raw foil bag at 2-8□ for storage.
10. Chromogen reagent is sensitive to light. Therefore it should be free of being exposed to light.
11. Kits out of validity should not be used in experiment.
12. The determination of test results must be subject to ELISA's readings. When using dual-wavelength for test, the wavelength should be set at 450nm and 630nm respectively.
13. All the samples, washing liquid and wastes should be treated as biowaste. Colour Reagent C should be 2M sulfuric acid and pay attention to safety when it is used.
14. Sample-adding at every step should be taken by adding instrument. Calibrate accuracy of the adding device to avoid experiment error. The time of single sample-adding should be controlled within 5 min. Just in case of exceeding samples, the volley for sample-adding is proposed.
15. Adhesive closures do not reuse or according to the experiments need to be cropped. Stick a strip of adhesive to compaction
16. Test determination and standard curve should be made at same time in every experiment, so there better be multiple pores. If the content of test sample were too high (OD value of the sample is higher than that of sample well maximum concentration), dilute to certain multiple by sample diluents (n times), then test the result and multiply it by dilution ratios when making calculation.
17. The sample containing NaN3 can't be tested because NaN3 inhibit the activities of horseradish peroxidase (HRP).
18. When washing board by plate washer, the volume of liquid injection to each well should be more than 350μl. Check if the sampling head is jammed. Yet the water absorption material with Paper Scrap should be cautiously used while washing board manually, free of the reaction between exogenous peroxidase analogues and chromogen reagents.
19. After the reaction being terminated by Colour Reagent C, read OD within 10 min.
20. During multiple pores experiment, the calculation result shall be mean value.
21. Sample hemolysis may cause false positive result, so this test is not appropriate for sample hemolysis.
22. During test, the strips should be put into closed box after adding samples and the humidity around should be kept at about 60%
23. It is advisable to check the thermostat by frequent calibration to keep its inside temperature at 37□. Ensure the experimental temperature being steady.
24. For 48T Elisa Kit, all components are 50% amount of 96T.
25. If there is any difference, the English instruction shall prevail.

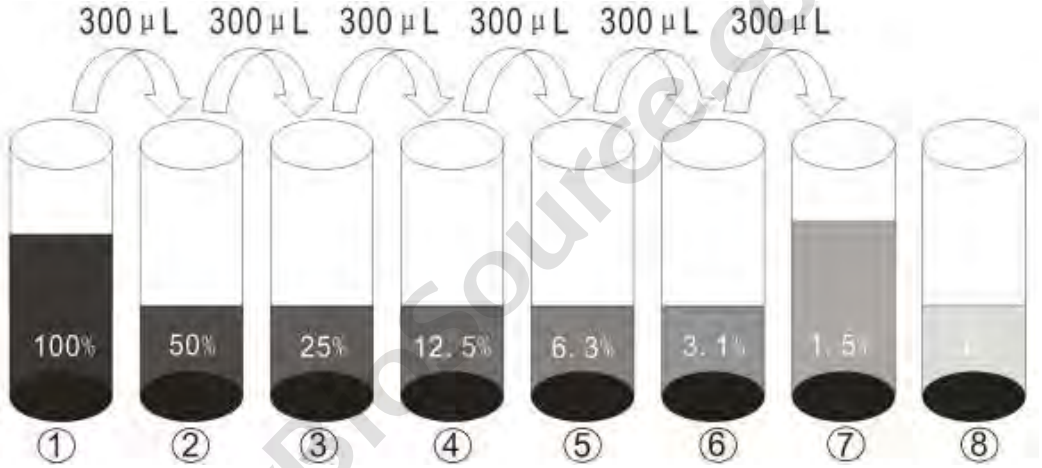
【Test preparation】

1.Please get the Elisa Kit out of refrigerator 20 minutes in advance and take test when

it balances to room temperature.

2. Dilute the concentrated washing solution with double distilled water (1:25). Put the unused back.
3. Human ADAM10 standard sample: Add diluent 1.0ml into human ADAM10 lyophilized standard sample and keep it still for 30 min. After the sample completely dissolved, mix it slightly and mark label on the tube□, then take dilution as needed.(It is recommended to using following concentration value to standard curve: 10, 5, 2.5, 1.25, 0.625, 0.312,0.156ng/ml). Note: Make sure the lyophilized standard completely dissolved and well mixed.
4. Legend of standard sample dilution method: Take 7 clean tubes and label them with ②,③,④,⑤,⑥,⑦,⑧respectively. Add 300μl standard sample diluent into each tube. Pipette out 300μl diluent from tube ①to tube ②and mix well. Further Pipette out 300μl diluent from tube ②to tube ③, and mix well. Repeat steps above up to tube ⑦. Standard sample dilution in tube⑧is negative control.

Note: The redissolved standard liquid（10 ng/ml）shall be discarded and not non-reusable.



Note: Reconstituted standard stock solution can not be reused.

5. Biotinylated human ADAM10 antibody liquid: Referring to needed amount, employ antibody diluent to dilute the concentrated biotinylated antibody (1:100) to form biotinylated antibody liquid. The preparation should be done 30 min in advance. And it's only for use on that day
6. Enzyme-conjugate liquid: Referring to needed amount, dilute the concentrated enzyme-conjugate by enzyme-conjugate diluent (1:100) to form enzyme-conjugate liquid. The preparation should be done 30 min in advance. And it's only for use on that day.
7. Colour Reagent liquid: Prepare Colour Reagent liquid 30 min in advance with Colour Reagent A and Colour Reagent B by the proportion of 9:1.

【Washing method】

1.Automatic plate-washing machine: The required amount of lotion is 350μl and the injection and extraction interval should be 20－30secs. Be well aware of the

operation instruction before putting the machine into practice.

2. Manual plate-washing machine: add 350μl lotion to each well and keep it still for 30secs. Shake individual wells as dry as you can and clean them with absorbent paper. During the plate-washing process, pay attention to the lotion-adding step to avoid contamination and well-jumping..

【Steps】

Read-if using two wavelength plate, empty holes can not; if using a read single wavelength plate , set the empty hole, empty holes in addition to color working liquid and TMB terminates other than liquid, without any reagent.

1.Take out needed strips from zip lock bag which balances to room temperature. The unused strips and desiccant should be put back into the sealed aluminum foil bag at 2-8℃ for storage.

2. Set aside blank wells (if dual-wavelength reading plate is used, the blank wells could be ignored)

3. Add samples or different concentration of human ADAM10 standard samples to corresponding wells (100μl for each well), 0ng/ml well should be filled with standard diluent. Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 90 min.

4. Prepare biotinylated human ADAM10 antibody liquid 30min in advance.

5. Wash the Elisa plate 3 times

6. Add the biotinylated human ADAM10 antibody liquid to each well (100μl for each). Seal reaction wells with adhesive tapes, hatching in incubator at 37℃ for 60 min.

7. Prepare enzyme-conjugate liquid 30min in advance.

8. Wash the Elisa plate 3 times

9. Add enzyme-conjugate liquid to each well except blank wells (100μl for each). Seal the reaction wells with adhesive tapes, hatching in incubator at 37℃ for 30 min.

10. Wash the Elisa plate 5 times.

11. Add 100μl Colour Reagent liquid to individual well (also into blank well), hatching in dark incubator at 37℃. When color for high concentration of standard curve become darker and color gradient appears, the hatching can be stopped. The chromogenic reaction should be controlled within 30 min.

12. Add 100μl Colour Reagent C to individual well (also into blank well). Mix well. Read OD（450nm）within 10 min.

【Result determination】

1. OD value of each sample and specimen should minus that of blank well (if not, the standard curve of zero well should intersect at Y axis)

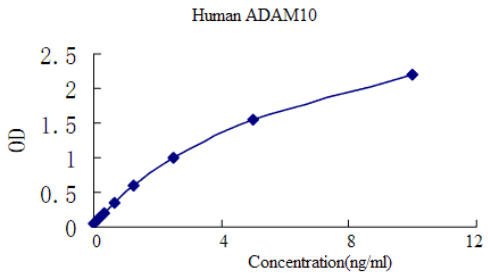
2. Draw standard curve manually. Take concentration value of samples as abscissa and OD readings as vertical coordinate. Use smooth line to connect each coordinate point of standard sample. The concentration of samples can be found by checking sample OD reading. It is recommended to employ the professional curve software

(e.g. curve expert 1.3) to analyze and compute the result.

3. If the sample OD is higher than the upper limit of standard curve, the sample should be re-diluted and the experiment rerun. Multiply the result by dilution factor when calculating the unknown.

Note: This chart is only for reference. The calculation of specimens' content shall be subject to the standard curve made for samples in same experiment.

【Reference curve】



Note: This chart is for reference only, should be based on the test standard dwarfed standard curve to calculate the specimen content.

【Summary of operating procedures】

Prepare reagents, samples and standards

↓

Add to the prepared sample and the standard reaction at 37 ℃ for 90 minutes

↓

Washe the plate ,adding a biotinylated antibody working solution, 37 ℃ for 60 minute

↓

Washed three times to add the Enzyme working solution, 37 ℃ for 30 minutes

↓

Washer five times, and then add the Colour Reagent solution, 37 ℃ within 30minutes

↓

Add to the Colour Reagent C

↓

Microplate reader measured OD values within 10 minutes

↓

Calculate the factor content of specimens tested

↓

Kit parameters

【Detection range】 10 ng/ml-0.156 ng/ml

【Sensitivity】the minimum detectable human ADAM10 up to 0.05ng/ml.

【Specific】No cross-reaction with other factors.

【Intra assay Precision】≤ 8%

【Inter assay Precision】≤ 12%

【Recovery】70 - 110 percent.
【Storage】-20℃ [Short-term should be placed 4℃(such as two weeks)]
【Uses】used in vitro quantitative analysis of liquid samples (Universal).
【Specifications】96T.
【Production Date】See microtiter plates aluminum foil bag sealing stamp.
【Validity】12 months (-20℃).
【Reference】

1. Moss ML, Bartsch JW (June 2004).
2. Nagano O, Saya H (December 2004).
3. Blobel CP (January 2005).
4. Janes PW, Saha N, Barton WA, Kolev MV, Wimmer-Kleikamp SH, Nievergall E, Blobel CP, Himanen JP, Lackmann M, Nikolov DB (October 2005).
5. Haass, C.; Kaether, C.; Thinakaran, G.; Sisodia, S. (2012).