ELISA PRODUCT INFORMATION & MANUAL

Human Adrenomedullin/ADM ELISA Kit (Chemiluminescence)

NBP2-66648

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Intended use

This CLIA kit applies to the in vitro quantitative determination of Human ADM concentrations in serum and plasma, please inquiry if your samples are other biological fluids.

Specification

- Sensitivity: 7.5pg/mL.
- Detection Range: 12.5-800pg/mL.
- Specificity: This kit recognizes Human ADM in samples. No significant cross-reactivity or interference between Human ADM and analogues was observed.
- Repeatability: Coefficient of variation is < 15%.

Test principle

This CLIA kit uses the Sandwich-CLIA principle. The micro CLIA plate provided in this kit has been pre-coated with an antibody specific to Human ADM. Standards or samples are added to the micro CLIA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human ADM and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human ADM, biotinylated detection antibody and Avidin-HRP conjugate will appear fluorescence. The Relative light unit (RLU) value is measured by the Chemiluminescence immunoassay analyzer. The RLU value is positively associated with the concentration of Human ADM. You can calculate the concentration of Human ADM in the samples by comparing the RLU value of the samples to the standard curve.

Kit components & Storage

An unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received

Item	Specifications	Storage
Micro CLIA Plate	96T: 8 wells ×12 strips 48T: 8 wells ×6 strips 24T: 8 wells ×3 strips	-20℃, 6 months
Reference Standard	96T: 2 vials 48T: 1 vial 24T: 1 vial	
Concentrated Biotinylated Detection Ab(100×)	96T: 1vial, 120μL 48T: 1vial, 60μL 24T: 1vial, 60μL	
Concentrated HRP Conjugate(100×)	96T: 1vial, 120μL 48T: 1vial, 60μL 24T: 1vial, 60μL	-20℃(Protect from light), 6 months
Reference Standard & Sample Diluent	1vial, 20mL	4℃, 6 months
Biotinylated Detection Ab Diluent	1vial, 14mL	
HRP Conjugate Diluent	1vial, 14mL	
Concentrated Wash Buffer (25×)	1vial, 30mL	
Substrate Reagent A	1vial, 5mL	4℃(Protect from light)
Substrate Reagent B	1vial, 5mL	4℃(Protect from light)
Desiccant	1 copy	
Plate Sealer	5pieces	
Manual	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other supplies required

Chemiluminescence immunoassay analyzer
High-precision transfer pipette, EP tubes and disposable pipette tips
Incubator capable of maintaining 37℃

Deionized or distilled water
Absorbent paper
Loading slot

Note

1. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
2. A freshly opened CLIA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results.
3. Do not reuse the reconstituted standard, biotinylated detection Ab working solution, concentrated HRP conjugate working solution. The unspent undiluted concentrated biotinylated detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.
4. Do not mix or use components from other lots.
5. Change pipette tips in between adding standards, in between sample additions, and in between reagent additions. Also, use separate reservoirs for each reagent.

Sample collection

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g 2 - 8℃. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable, non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8 ℃ within 30 min of collection. Collect the supernatant to carry out the assay. Hemolyzed samples are not suitable for CLIA assay!

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 μL of pre-cooled PBS to keep the cells suspended. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500×g at 4 ℃. Remove the cell fragments, collect the supernatant to carry out the assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolyzed blood may

affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

Cell culture supernatant or other biological fluids: Centrifuge samples for 20 minutes at 1000xg at 2 - 8℃. Collect the supernatant and carry out the assay immediately.

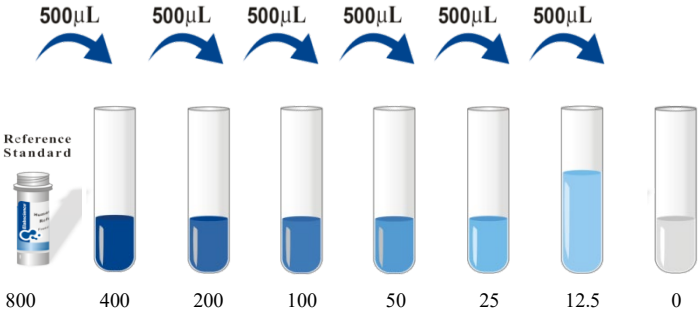
Note for sample

1. Samples should be used within 7 days when stored at 4℃, otherwise samples must be divided up and stored at -20°C (≤1month) or -80°C (≤3months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility to cause a deviation due to the introduced chemical substance.
5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use.
2. **Wash Buffer:** Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 800 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 800、400、200、100、50、25、12.5、0 pg/mL.
   Dilution method: Take 7 EP tubes, add 500uL of Reference Standard & Sample Diluent to each tube. Pipette 500μL of the 800pg/mL working solution to the first tube and mix

up to produce a 400pg/mL working solution. Pipette 500μL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



4. **Biotinylated Detection Ab working solution**: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the stock tube before use, dilute the 100× Concentrated Biotinylated Detection Ab to 1×working solution with Biotinylated Detection Ab Diluent.
5. **Concentrated HRP Conjugate working solution**: Calculate the required amount before the experiment (100 μL/well). In preparation, slightly more than calculated should be prepared. Dilute the 100× Concentrated HRP Conjugate to 1× working solution with Concentrated HRP Conjugate Diluent.
6. **Substrate Mixture Solution:** Calculate the required amount before the experiment (100μL/well). In preparation, slightly more than calculated should be prepared. Mix the Substrate Reagent A and B with equal volumes before use. Note: don't open the vial until you need it.

Assay procedure (A brief assay procedure is on the 11th page)

1. Add the **Standard working solution** to the first two columns: Each concentration of

the solution is added in duplicate, to one well each, side by side (100 μL for each well). Add the samples to the other wells (100 μL for each well). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37℃. Note: solutions should be added to the bottom of the micro CLIA plate well, avoid touching the inside wall and causing foaming as much as possible.

2. Remove the liquid out of each well, don't wash. Immediately add 100μL of **Biotinylated Detection Ab working solution** to each well. Cover with the Plate sealer. Gently mix up. Incubate for 1 hour at 37℃.
3. Aspirate or decant the solution from each well，add 350μL of **wash buffer** to each well. Soak for 1~2 minutes and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times in total. Note: a microplate washer can be used in this step and other wash steps.
4. Add 100μL of **HRP Conjugate working solution** to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37℃.
5. Aspirate or decant the solution from each well, repeat the wash process for five times as conducted in step 3.
6. Add 100μL of **Substrate Mixture Solution t**o each well. Cover with a new plate sealer. Incubate for not more than 5 minutes at 37℃. Protect the plate from light.
7. Determine the RLU value of each well at once.

Calculation of results

Average the duplicate readings for each standard and samples, then subtract the average zero standard RLU. Plot a four parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and RLU values on the y-axis.

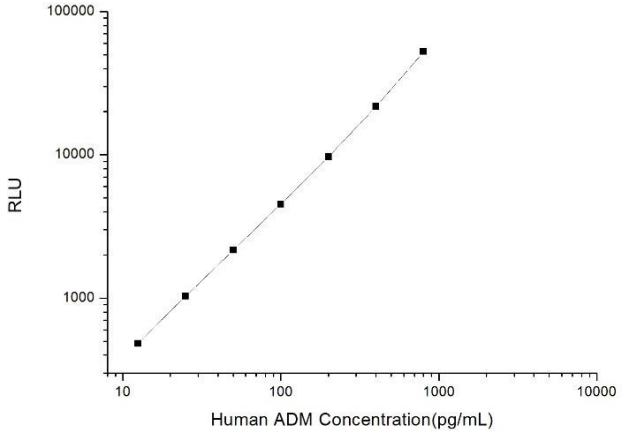
If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the RLU of the sample surpasses the upper limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the RLU values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or

temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.

Concentration(pg/mL)	800	400	200	100	50	25	12.5	0
RLU 1	52065	22137	10954	4575	2337	1145	519	28
RLU 2	60677	25087	9754	5109	2161	1097	521	30
Average RLU	56371	23612	10354	4842	2249	1121	520	29
Corrected RLU	56342	23583	10325	4813	2220	1092	491	---



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid range and high level Human Adrenomedullin/ADM were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid range and high level Human ADM were tested on 3 different plates, 20 replicates in each plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean(pg/mL)	43.82	84.72	300.61	47.63	80.1	314.32
Standard deviation	4.6	7.48	21.01	5.59	7.42	31.68
CV (%)	10.5	8.83	6.99	11.74	9.26	10.08

Recovery

The recovery of Human Adrenomedullin/ADM spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum (n=5)	100-115	108
EDTA plasma (n=5)	86-101	92
Cell culture media (n=5)	90-104	95

Linearity

Samples were spiked with high concentrations of Human Adrenomedullin/ADM and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.

		Serum (n=5)	EDTA plasma (n=5)	Cell culture media (n=5)
1:2	Range (%)	91-106	98-111	101-116
	Average (%)	96	105	107
1:4	Range (%)	97-109	92-105	87-98
	Average (%)	104	98	93
1:8	Range (%)	96-112	87-102	100-117
	Average (%)	102	94	108
1:16	Range (%)	89-100	93-106	91-107
	Average (%)	95	99	98

Troubleshooting

If the results are not good enough, please take pictures and save the experimental data in time. Keep the used plate and remaining reagents. Then contact our technical support to solve the problem. Meanwhile, you could also refer to the following materials:

Problem	causes	solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by gentle mixing.
	Wells are not completely aspirated	Completely aspirate wells between steps.
Low fluorescence	Insufficient incubation time	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	
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of target protein is 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	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the CLIA kit	All the reagents should be stored according to the instructions.
	Too long incubation time	Ensure precise incubation time.

SUMMARY

1. Add 100μL standard or sample to each well. Incubate 90mintues at 37°C

2. Remove the liquid. Add 100μL Biotinylated Detection Ab. Incubate 1 hour at 37°C

3. Aspirate and wash 3 times

4. Add 100μL HRP Conjugate. Incubate 30 minutes at 37°C

5. Aspirate and wash 5 times

6. Add 100μL Substrate Mixture Solution. Incubate 5 minutes at 37°C

7. Determine the RLU value

8. Calculation of results

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the CLIA immunoassay, the possibility of interference cannot be excluded.
3. The final experimental results will be closely related to the validity of products, operational skills of the operators, the experimental environments and so on. We are only responsible for the kit itself, but not for the samples consumed during the assay. The users should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
4. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions.
5. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Chemiluminescence immunoassay analyzer. Please read the instructions carefully and adjust the instrument prior to the experiment.
6. Even the same operator might get different results in two separate experiments. In order to get reproducible results, the operation of every step in the assay should be controlled.
7. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might arise from the above reasons too.
8. Kits from different manufacturers or other methods for testing the same analyte could bring out inconsistent results, since we haven't compared our products with those from other manufacturers.
9. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.