

USER MANUAL



ELISA KIT

ELISA Kit for Adiponectin (ADP)

Catalog No. tcue1485

96 Tests

FOR RESEARCH USE ONLY!

Please read completely user manual and storage condition.

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Available Sizes

Size: 96 Tests



Specifications

Research Area:	Endocrinology Reseach
Species Reactivity:	Mouse (Mus musculus)
Sample Type:	Serum, plasma and Tissue Homogenates, other biological fluids
Sensitivity:	14.2pg/mL
Recovery:	Recovery ranges are from 93-105% .
Detection Range:	31.2-2000pg/mL
Assay Time:	3 hrs
Detection Method:	Sandwich ELISA, Double Antibody,Colorimetric; absorbance at 450 nm
Precision:	Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Adiponectin (ADP) were tested 20 times on one plate, respectively. Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level ADP were tested on 3 different plates, 8 replicates in each plate. Intra-assay coefficient of variation (CV) <8%. Inter-assay CV <10%.

Test Principle

The microplate provided in this kit has been pre-coated with an antibody specific to erythropoietin. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to erythropoietin. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain erythropoietin, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm \pm 10nm. The concentration of erythropoietin in the samples is then determined by comparing the O.D. of the samples to the standard curve.

Materials and Storage.

1. For unused kit: All the reagents should be kept according to the labels on vials. The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon receipt while the others should be at 4 °C .
2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.

Components

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	4
Standard	2	Standard Diluent	1×20mL
Detection Reagent A	1×120μL	Assay Diluent A	1×12mL
Detection Reagent B	1×120μL	Assay Diluent B	1×12mL
TMB Substrate	1×9mL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000μL
- Multi-channel pipette calibrated to deliver 50-250μL
- Plate shaker
- Disposable tips
- Microcentrifuge tubes
- Vortex-Mixer
- Distilled or de-ionized water
- 0.01 mol/L (or 1X) Phosphate Buffered Saline (PBS). PH 7.0-7.2
- Microplate reader capable of reading 450nm.

Safety Precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
- Disposal must be performed in accordance with local regulations.
- Only trained laboratory personnel should execute this test.

The Stop Solution suggested for use with this kit is an acid solution.

Wear eye, hand, face, and clothing protection when using this material.

Sample Collection and Storage

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant.

Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection.

Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use.

Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (catalog: IS007, different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders works, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifugated for 5 minutes at 10,000×g.

Remove the supernatant and assay immediately or aliquot and store at ≤-20°C

Cell Lysates - Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 10⁷ cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris.

Assay immediately or aliquot and store at ≤-20°C

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Reagent Preparation and Storage

Bring all reagents to room temperature before use.

1. Wash Solution:

Dilute 20mL of Concentrated Wash Solution (30X) into 580 mL with deionized or distilled water to prepare 600mL of wash Solution (1x)

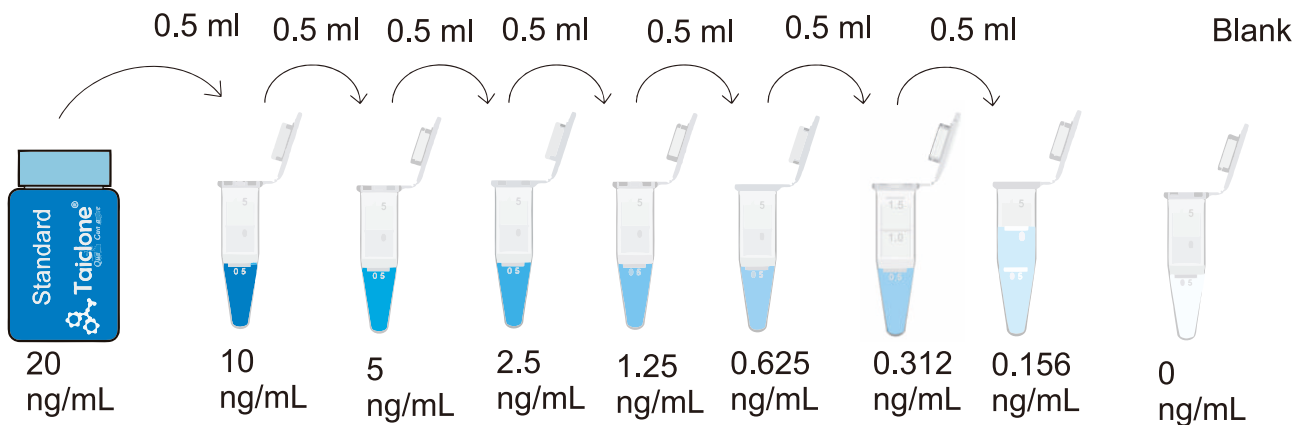
Put unused solution back at 4°C. If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

2. Standard:

Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 20 ng/mL. Please firstly dilute the stock solution to 2,000pg/mL and the diluted standard serves as the highest standard (2,000pg/mL).

Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer.

Set up 7 points of diluted standard such as 10ng/mL, 5ng/mL, 2.5ng/mL, 1.25ng/mL, 0.625ng/mL, 0.312ng/mL, 0.156ng/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.



3. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with **Assay Diluent A and B**, respectively.
4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standards within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.
3. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL for one pipetting.
4. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
5. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

Sample Preparation:

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. Serum/plasma samples require about a 5,000 fold dilution. For example, to prepare a 1:5,000 dilution of sample, transfer 10µL of sample to 490µL PBS. This yields a 1:50 dilution. Next, dilute the 1:50 sample by transferring 10µL to 990µL PBS. You now have a 1:5,000 dilution of your sample. Mix thoroughly at each stage. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.
6. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
7. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernatant may not be detected by the kit.
8. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Assay Procedure:

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100µL of **Detection Reagent A working solution** to each well, cover the wells with the plate sealer and incubate for 1 hour at 37 °C.
4. Aspirate the solution and wash with 350µL of (1x) Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100µL of **Detection Reagent B working solution** to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37 °C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.
7. Add 90µL of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37 °C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. Add 50µL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:

1. **Assay preparation:** Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20oC.
2. **Samples or reagents addition:** Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
5. **Controlling of reaction time:** Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. **TMB Substrate** is easily contaminated. Please protect it from light.
7. The environment humidity which is less than 60% might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.

Summary

1. Prepare all reagents, samples and standards.
2. Add 100 μ L standard or sample to each well for 60 minutes at 37°C
3. Remove the liquid of each well. DO NOT WASH.
4. add 100 μ L Detection Reagent A working solution to each well , Cover well, incubate 60 mins at 37°C
5. Aspirate solution and wash 3 times 350uL of washing buffer
6. Add 100 μ L Detection Reagent B solution to each well. Cover wells, Incubate for 30 mins at 37°C
7. Aspirate and wash 5 times as step 5
8. Add 90 μ L Substrate Solution Incubate 10 -20 minutes at 37°C
9. Add 50 μ L Stop Solution. Read at 450nm immediately
10. Calculation of results

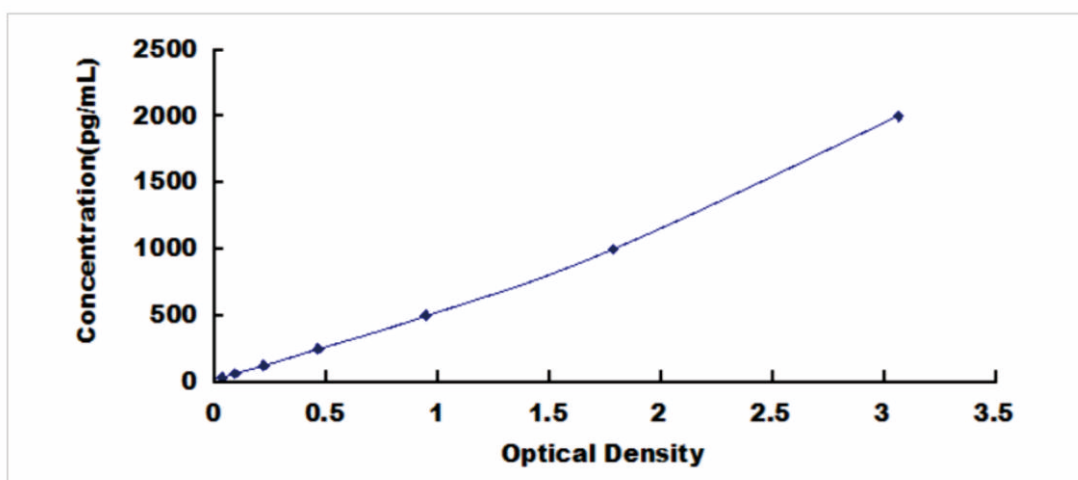
Calculation of result.

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Construct a standard curve by plotting the mean O.D. and concentration for each standard and draw a best fit curve through the points on the graph or create a standard curve on log-log graph paper with erythropoietin concentration on the y-axis and absorbance on the x-axis. Using some plot software, for instance, curve expert 1.30, is also recommended.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data & Standard Curve

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is the dependent variable. However, the O.D. values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), plotting log of the data to establish standard curve for each test is recommended. Typical standard curve below is provided for reference only.



Typical Standard Curve for Adiponectin, Mouse ELISA.

Recovery

Matrices listed below were spiked with certain level of recombinant adiponectin and the recovery rates were calculated by comparing the measured value to the expected amount of adiponectin in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	82-98	92
EDTA plasma(n=5)	96-105	101
heparin plasma(n=5)	80-92	86

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of adiponectin and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1: 2	1: 4	1: 8	1: 16
serum(n=5)	97-104%	80-96%	92-101%	85-92%
EDTA plasma(n=5)	82-98%	84-91%	83-103%	92-105%
heparin plasma(n=5)	88-102%	79-93%	86-99%	87-97%

Samples were diluted prior to assay as described in the SAMPLE PREPARATION section.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level adiponectin were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level adiponectin were tested on 3 different plates, 8 replicates in each plate. $CV(\%) = SD/mean \times 100$

Intra-Assay: $CV < 10\%$

Inter-Assay: $CV < 12\%$

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% prior to the expiration date under appropriate storage condition.

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly monitored.

It is also strongly suggested that the assay is performed by the same operator from the beginning to the end.

Sample Value

Serum/Plasma - Samples from specific pathogen free (SPF) mouse were evaluated in this assay.

Sample	Range($\mu\text{g/mL}$)	Detectable(%)
Serum (n=25)	1.1-7.5	100
EDTA plasma (n=20)	0.8-6.3	100
Heparin plasma (n=20)	0.9-6.5	100

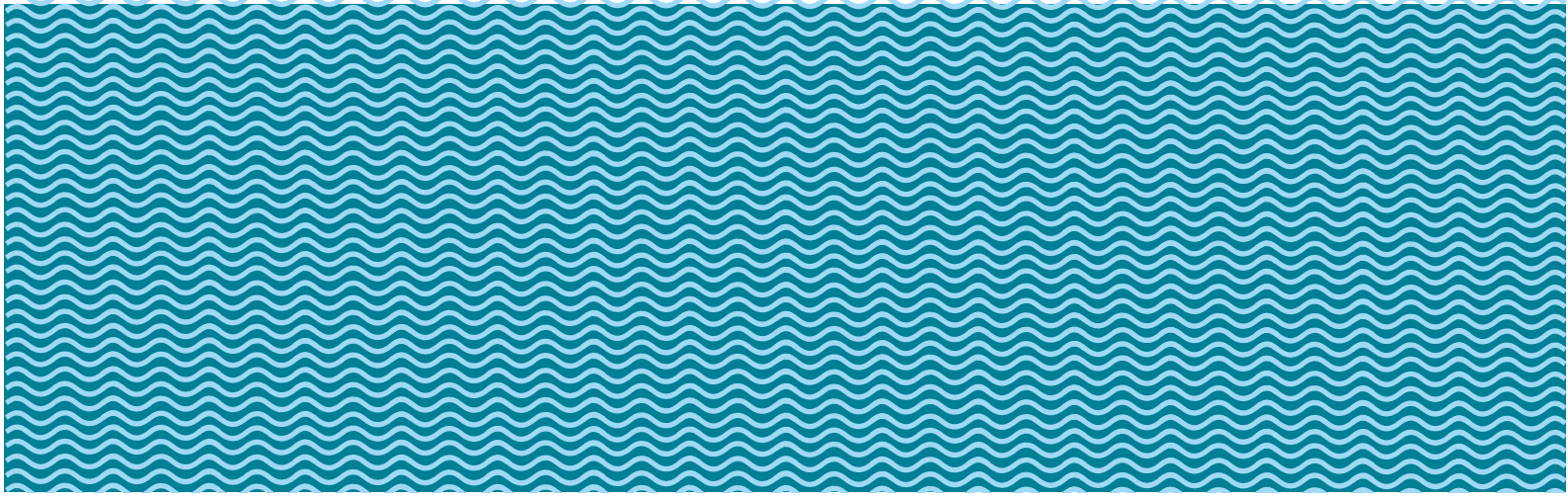
These data are our in-house data, only for reference.

Important Note

1. Limited by the current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
2. The final experimental results will be closely related to validity of the products, so the kit should be used prior to the expiration date. And please store the kits exactly according to the instruction.
3. 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 instruction attached in kit while electronic ones from our website is only for reference.
4. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
5. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism. TMB Substrate should remain colorless till it is reacted with the enzyme which binds to the microplate.
6. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microplate from the storage bag until needed.
7. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. at $450 \pm 10\text{nm}$ wavelength is acceptable for use in absorbance measurement. Please read the instruction carefully and adjust the instrument prior to the experiment.
8. Variation in sample preparation and each step of experimental operation may cause different results. In order to get better reproducible results, the operation of each step in the assay should be controlled.
9. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
10. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.
11. The standard of the kit and immunogen used for antibody preparation are commonly recombinant proteins, as different fragments, expression systems, purification methods might be used in recombinant protein preparation, we can not guarantee the kit could detect recombinant protein from other companies. So, it is not recommended to use the kit for the detection of recombinant protein.
12. Please predict the concentration of target molecules in samples, or arrange a preliminary experiment, it is a good way to solve specific problem, e.g. the concentration of samples are beyond the detection range of the kit.
13. The kit might not be suitable for detection of samples from some special experiment, for instance, knock-out experiments, due to their uncertainty of effectiveness.
14. The instruction manual is also for the kit of 48T, but all reagents of 48T kit are reduced by half.

Trouble Shooting

Problem	Possible Source	Correction Action
Poor Standard Curve	Improper standard curve preparation	Ensure accurate operation of the dilution
	Incomplete washing and aspiration	Adequate washing and adequate aspiration
	Inaccurate Pipetting	Check and Calibrate pipettes
Poor Precision	Incomplete washing of wells	Ensure sufficient washing
	Inadequate mixing and aspiration reagents	Adequate aspiration and mixing reagents
	Reused pipette tips, containers and sealers	Change and use new pipette tips, containers and sealers
	Inaccurate Pipetting	Check and Calibrate pipettes
Low O.D Values	Inadequate reagent volumes added to wells	Calibrate pipettes and Add adequate reagents
	Incorrect incubation times	Ensure sufficient incubation times
	Incorrect incubation temperature	Reagents balanced to room temperature
	Conjugate or substrate reagent failure	Mix conjugate & substrate, color should develop immediately
	No stop solution added	Follow the assay protocol in the kit manual
	Read beyond suggested reading time	Read within the time recommended in the manual
Sample Values	Improper Sample Storage	Store the sample properly and use the fresh sample
	Improper sample collection and preparation	Take proper sample collection and preparation method
	Low quantity of analyte in samples	Use new sample and repeat assay



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