ACE Biolabs

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Datasheet

Ver.1 Date : 20190328

Mouse IgG (Immunoglobulin G) ELISA Kit

Cat# E0014

Storage at 4˚C for 1 month

INTRODUCTION

This ELISA kit is designed for the quantitative determination of **mouse immunoglobulin G (IgG)** concentrations in serum, plasma, cell culture supernates, urine, and tissue homogenates.

SPECIFICATION

Sensitivity: 29 ng/mL.

Detection Range: 125 – 8000 ng/mL

Specificity: This kit recognizes mouse IgG in samples. No significant cross-reactivity or interference between mouse IgG and analogues was observed.

Intra-assay Precision (Precision within an assay): CV% <8%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV% <10%

Three samples of known concentration were tested in twenty assays to assess.

PRINCIPLE of KIT

This kit employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with mouse IgG. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for mouse IgG. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in opposite to the amount of mouse IgG in samples. The color development is stopped and the intensity of the color is measured.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1 (96 wells)
Standard	6 x 0.5 ml
HRP-conjugate	1 x 6 ml
Sample Diluent	2 x 20 ml
Wash Buffer (25 x concentrate)	1 x 20 ml
TMB Substrate	1 x 10 ml
Stop Solution	1 x 10 ml
Adhesive Strip (For 96 wells)	4

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ng/ml)	0	125	500	1000	2000	8000

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. Do not use the kit beyond the expiration date</td></tr>
<tr><td rowspan="7">Opened kit</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C.
Try to keep it in a sealed aluminum foil bag, and avoid the damp.</td></tr>
<tr><td>HRP-conjugate</td><td rowspan="6">May be stored for up to 1 month at 2 - 8°C.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

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

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm. An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100ml and 500ml graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

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 ELISA Plate may appear to have 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. The microplate reader should have a 450 (±10 nm) filter installed and a detector that can detect the wavelength.

The optical density should be within 0~3.5.

5. Do not mix or use components from other lots.
6. 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˚C before centrifugation for 15 min at 1000 x g at 2~8 C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be non-endotoxin.

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

Cell culture supernatants: Remove particulates by centrifugation for 15 minutes at 1000 x g, 2 - 8°C and assay immediately.

Tissue homogenates: 100 mg tissue was rinsed with 1X PBS, homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g, 2 - 8°C. The supernate was removed and assayed immediately.

Urine: Use a sterile container to collect urine samples. Remove any particulates by centrifugation at 1000 x g for 15 minutes, 2 - 8°C and assay immediately. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION:

Recommend to dilute the serum or plasma samples with Sample Diluent (1:10000) before test. The suggested 10000-fold dilution can be achieved by adding 3 µl sample to 297 µl of Sample Diluent. Complete the 10000-fold dilution by adding 3 µl of this solution to 297 µl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments

NOTE for SAMPLE:

1. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
2. Samples should be assayed within 5 days when stored at 2-8°C, otherwise samples must be aliquot and stored at -20°C (≤ 1 month) or -80°C (≤ 2 months). Avoid repeated freeze-thaw cycles.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. 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.
5. If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.
6. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation due to the introduced chemical substance.
7. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection

antibody.

REAGENT PREPARATION

1. Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
2. Bring all reagents to room temperature (18~25°C) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
3. **Wash Buffer (1 x):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).
4. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettes are calibrated. It is recommended to suck more than 10 µl for once pipetting.
5. Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a **Blank** well without any solution.
4. Add 50 µl of **Standard** or **Sample** per well. Standard need test in duplicate.
5. Add 50 µl of **HRP-conjugate** to each well immediately (not to Blank well).
6. Mix well and then incubate for 60 minutes at 37°C. A plate layout is provided to record standards and samples assayed.
7. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with **Wash Buffer** (200 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 2 minutes, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 90 µl of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. **Protect from light**.
9. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

***Samples may require dilution. Please refer to Sample Preparation section.**

NOTE for ASSAY:

1. **Samples or reagents addition:** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. 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 each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. **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 have been added to the well strips, **DO NOT** let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
3. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential to 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 falsely elevated absorbance reading. When using an automated plate washer, adding a 2 minutes soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
4. **Controlling of reaction time:** Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
5. **TMB Substrate is easily contaminated.** TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
6. **Stop Solution should be added to the plate in the same order as the TMB Substrate.** The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

CALCULATION of RESULTS

Average the duplicate readings for each standard and sample and subtract the average optical density of Blank.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse IgG concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TROUBLESHOOTING

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 in between steps.
Low signal	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	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB, rapid coloring.
Deep color but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Open the Microplate Reader ahead to pre-heat.
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 ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

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. The final experimental results will be closely related to the validity of products, operational skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions!

4. Incorrect results may occur because of incorrect operations during the reagents preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.
5. 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.
6. 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 equipments, and so on. Intra-assay variance among kits from different batches might arise from the above reasons, too.