IMMUNOPORT

SKU IP400111

ELISA KIT FOR CHICKEN IGG

Research Use Only
For *in vitro* applications - not for consumption

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VERSION 1.0

INTENDED USE

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement of IgG in Chicken (Gallus) serum, plasma and other biological fluids.

KIT CONTENTS & STORAGE

Pre-coated Microplate [12 X 8 Strips]
Standard [2 vials]
Standard Diluent [20mL]
Detection Reagent A and B [120µL each]
Assay Diluent A and B [12mL each]
30X Wash solution [20mL]
TMB Substrate [9mL]
Stop Solution [6mL]
Plate Sealing Membrane [4pcs]
User manual [1pc]
Zipper bag [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C while the others could be at 4°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37 °C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

TEST PRINCIPLE

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to IgG has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotin labeled IgG and unlabeled IgG (Standards or samples) with the pre-coated antibody specific to IgG. After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of IgG in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of IgG in the sample.

CHARACTERISTICS

Sample Type: Serum, plasma, and other biological fluids.

Detection Range: 246.9-20,000ng/mL
Minimum Detection Dose: 94.7ng/mL
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%

Cross Reactivity: None observed with analogues
Expiry: As per kit label
Storage (Unopened kit): -20°C in shelf life, 4°C (for upto a month)

Once opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8°C.

LINEARITY

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IgG 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)	88-101%	93-106%	86-103%	89-98%
EDTA plasma (n=5)	87-104%	80-97%	82-96%	84-99%
Heparin plasma (n=5)	90-99%	81-92%	92-105%	80-101%

RECOVERY

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

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	79-92	85
EDTA plasma (n=5)	80-97	92
Heparin plasma (n=5)	93-104	99

SAMPLE COLLECTION

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 1000×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.

Cell Culture Supernates and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

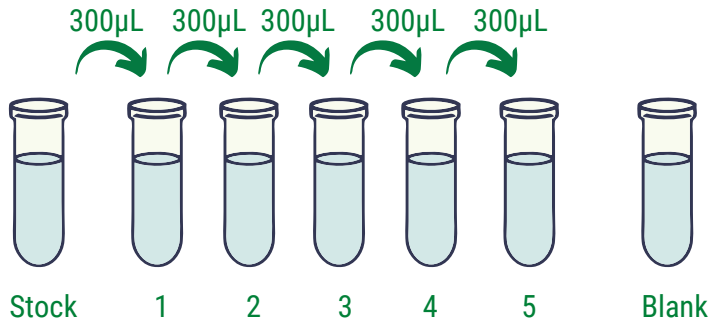
Note:

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 detected.
3. When performing the assay, bring samples to room temperature.
4. Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.
5. It is highly recommended to use serum instead of plasma.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

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 60,000ng/mL. Please firstly dilute the stock solution to 20,000ng/mL and the diluted standard serves as the highest standard (20,000ng/mL). Then prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 20,000ng/mL, 6,666.7ng/mL, 2,222.2ng/mL, 740.7ng/mL, 246.9ng/mL, and the last EP tubes with Standard Diluent is the blank as 0ng/mL.



Concentrations

Stock- 60,000ng/mL
1- 20,000ng/mL
2- 6,666.7ng/mL
3- 2,222.2ng/mL
4- 740.7ng/mL
5- 246.9ng/mL
Blank- 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 standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.

3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.

4. 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.

5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.

6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.

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 2,000 fold dilution. For example, to prepare a 1:2,000 dilution of sample, transfer 20µL of sample to 380µL PBS. This yields a 1:20 dilution. Next, dilute the 1:20 sample by transferring 10µL to 990µL PBS. You now have a 1:2,000 dilution of your sample. Mix thoroughly at each stage. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).

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 supernates 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.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50µL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50µL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37°C . Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

2. 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. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

3. Add 100µL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37°C after covering it with the Plate sealer.

4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.

5. 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.

6. 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.

7. 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 -20°C.

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.

CALCULATION

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between IgG concentration in the sample and the assay signal intensity.

Average the duplicate readings for each standard, control, and samples. Create a standard curve with the log of IgG concentration on the y-axis and absorbance on the x-axis. Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

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