Rat IgG ELISA Kit

【Catalog No】EK371

【SIZE】48T/96T

【 INTENDED USE 】For the quantitative determination of rat Immunoglobulin G (IgG) concentration in cell culture supernates, serum and plasma.

【INTRODUCTION】

Immunoglobulin G (IgG) is a type of antibody. It is a protein complex composed of four peptide chains — two identical heavy chains and two identical light chains arranged in a Y-shape typical of antibody monomers. Each IgG has two antigen binding sites. IgG is the main type of antibody found in blood and extracellular fluid allowing it to control infection of body tissues. IgG molecules are created and released by plasma B cells. IgG is the only isotype that has receptors to facilitate passage through the human placenta, thereby providing protection to the fetus in utero.

IgG antibodies are extracted from donated blood plasma and used as a therapeutic known as intravenous immunoglobulin (IVIG). This is used to treat immune deficiencies, autoimmune disorders, and infections.

【PRINCIPLE OF THE ASSAY】

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat IgG has been pre-coated onto a microplate. Standard, samples and HRP-linked detect antibody specific for IgG are pipetted into the wells and IgG present is bound by the immobilized antibody and detect antibody following incubation. After washing, substrate solution is added to the wells and color develops in proportion to the amount of IgG bound in the initial step. The color development is stopped and the intensity of the color is measured.

【MATERIALS PROVIDED】

PART	PART #	EK371-48	EK371-96
Coated Microplate	EK371P	48T	96T
Standard	EK371S	1 vial	2 vials
Detect antibody	EK371D	1 vial	1 vial
Assay Buffer (10×)	E0310	20ml	20ml
TMB	E0230	6ml	11ml
Stop Solution	E0300	11ml	11ml
Washing Buffer (20×)	E0281	50ml	50ml
Adhesive Film	E0200	6	6

Note: Components from reagent kits of different batch numbers must not be used interchangeably.

OTHER SUPPLIES REQUIRED

1) Microplate reader capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
2) Pipettes and pipette tips.
3) 50 µl to 300 µl adjustable multichannel micropipette with disposable tips.
4) Multichannel micropipette reservoir.
5) Beakers, flasks, cylinders necessary for preparation of reagents.
6) Deionized or distilled water.
7) Polypropylene test tubes for dilution.

【STORAGE】

Store at 2-8℃; refer to the kit label for expiration date.

For opened kits:

Pre-coated microplate: Can be stored at 2-8℃ for approximately 1 month (return unused strips to the aluminum foil bag and reseal).

Standard: Can be stored at -20℃ for approximately 1 month (discard after single-use reconstitution).

Other components: Can be stored at 2-8℃ for approximately 1 month.

【SAMPLE COLLECTION AND STORAGE】

1) **Cell Culture Supernates** - Remove particulates by centrifugation and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

2) **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1,000 × g. Remove serum and assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

3) **Plasma** - Collect plasma using EDTA, citrate or heparin as anticoagulant. Centrifuge at 1,000 × g within 30 minutes of collection. Assay freshly prepared samples immediately or aliquot and store samples at ≤ -20℃ for later use. Avoid repeated freeze-thaw cycles.

4) Other biological samples might be suitable for use in the assay. Cell culture supernates, serum and plasma were tested with this assay. Dilution with Assay Buffer may be needed.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

If samples are to be run within 24 hours, they may be stored at 2 to 8℃. For longer storage, aliquot samples and store frozen at -20℃. Avoid repeated freeze-thaw cycles.

【SAMPLE PREPARATION】

Normal serum and plasma samples require a 200,000-fold dilution. A suggested 200,000-fold dilution is three step dilution: first, 10 µl sample + 190 µl Assay Buffer (1×); next, 10 µl Mix I + 990 µl Assay Buffer (1×); then, 10 µl Mix II + 990 µl Assay Buffer (1×).

【REAGENT PREPARATION】

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1,000 ml graduated cylinder. Bring to final volume of 1,000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25℃. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder. Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8℃. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1：100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Sample Dilution: If your samples have high **IgG** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

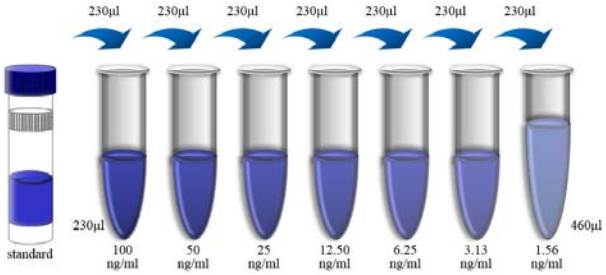
Rat IgG Standard: Reconstitute **Rat IgG Standard** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and

homogeneous solubilization (concentration of reconstituted standard = 200 ng/ml).

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

Use polypropylene tubes.

- **For serum/plasma samples,** mixing concentrated Rat IgG standard (230 µl) with 230 µl of Assay Buffer (1×) creates the high standard (100 ng/ml). Pipette 230 µl of Assay Buffer (1×) into each tube. Use the high standard to produce a 1:1 dilution series (scheme below). Mix each tube thoroughly before the next transfer. Assay Buffer (1×) serves as the zero standard (0 pg/ml).
- **For cell culture supernates,** mixing concentrated Rat IgG standard (230 µl) with 230 µl of cell culture medium creates the high standard (100 ng/ml). Pipette 230 µl of cell culture medium into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. Cell culture medium serves as the zero standard (0 pg/ml).



【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1) Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.

2) Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.

3) Add 300 µl Washing Buffer (1×) per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Washing Buffer (1×). Use the microwell strips immediately after washing. **Do not allow wells to dry.**

4) Add 100 µl 2-fold diluted Standard to Standard well. Add 100 µl Assay Buffer (1×)/ culture medium to Blank well.

5) **Serum/Plasma**: Add 100 µl of prediluted sample to the sample well. **Cell culture supernates**: Add 100 µl cell culture supernates to the sample well.

6) Add 50 µl of diluted Detect Antibody to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.

7) **Seal the plate with an adhesive film.** Incubate at room temperature (25°C±3°C) for 2 hours on a microplate shaker set at 300 rpm.

8) Aspirate each well and wash by filling each well with 300 µl Washing Buffer (1×), repeat five times for a total six washes. Complete removal of liquid at each step is essential to the best performance. After the last wash, remove any remaining Washing Buffer (1×) by aspirating or decanting. Invert the plate and tap it against clean paper towels.

9) Add 100 µl of Substrate Solution to each well. Incubate for 5 - 30 minutes at room temperature(25°C±3°C). **Protect from light.**

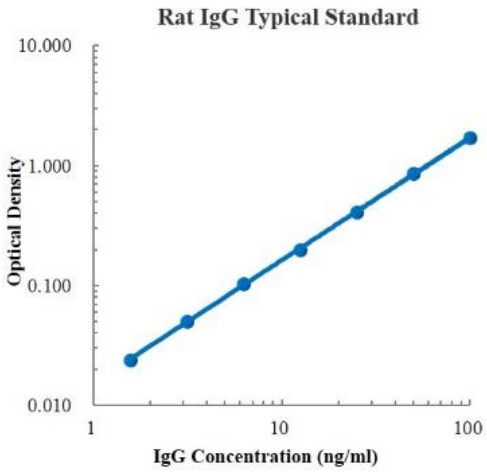
10) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11) Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

【TYPICAL DATA】

A standard curve must be run within each assay. The following standard curve is provided for demonstration only.

ng/ml	O.D.		Average	Corrected
0.00	0.036	0.034	0.035	
1.56	0.062	0.056	0.059	0.024
3.13	0.087	0.083	0.085	0.050
6.25	0.135	0.141	0.138	0.103
12.50	0.231	0.233	0.232	0.197
25.00	0.450	0.446	0.448	0.413
50.00	0.897	0.893	0.895	0.860
100.00	1.738	1.732	1.735	1.700



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