MULTI SCIENCES

Rat IgA ELISA Kit

【Catalog No】EK374

【SIZE】48T/96T

【 INTENDED USE 】For the quantitative determination of rat Immunoglobulin A (IgA) concentrations in cell culture supernates, serum and plasma.

【INTRODUCTION】

Immunoglobulin A (IgA) is an antibody that plays a critical role in mucosal immunity. Its heavy chains are of the type α. IgA has two subclasses (IgA1 and IgA2) and can exist in a dimeric form called secretory IgA (sIgA). In its secretory form, IgA is the main immunoglobulin found in mucous secretions, it is also found in small amounts in blood. The secretory component of sIgA protects the immunoglobulin from being degraded by proteolytic enzymes, thus sIgA can survive in the harsh gastrointestinal tract environment and provide protection against microbes that multiply in body secretions. sIgA can also inhibit inflammatory effects of other immunoglobulins. IgA is a poor activator of the complement system, and opsonises only weakly.

【PRINCIPLE OF THE ASSAY】

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

【MATERIALS PROVIDED】

PART	PART #	EK374-48	EK374-96
Coated Microplate	EK374P	48T	96T
Standard	EK374S	1 vial	2 vials
Detect antibody	EK374D	1 vial	1 vial
Assay Buffer (10×)	E0310	10ml	10ml
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 1,000-fold dilution. A suggested 1,000-fold dilution is two step dilution: first, 10 µl sample + 190 µl Assay Buffer (1×); next, 10 µl Mix + 490µl 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.

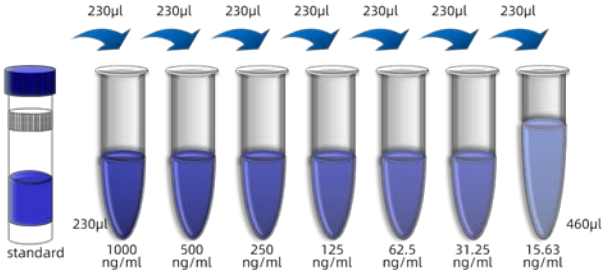
Sample Dilution: If your samples have high **IgA** content, dilute serum/plasma samples with Assay Buffer (1×). For cell culture supernates, dilute with cell culture medium.

Rat IgA Standard: Reconstitute **Rat IgA 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 = 2000 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 IgA standard (230 µl) with 230 µl of Assay Buffer (1×) creates the high standard (1000 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 IgA standard (230 µl) with 230 µl of cell culture medium creates the high standard (1000 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.Ensure reagent addition in step 4, 5 is uninterrupted and completed within 15 minutes.

6) **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.

7) 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.

8) Add 100 µl of diluted Detect Antibody to each well.

9) **Seal the plate with a fresh adhesive film.** Incubate at room temperature (25°C±3°C) for 1 hour on a microplate shaker set at 300 rpm.

10) Repeat aspiration/wash as in step 7.

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

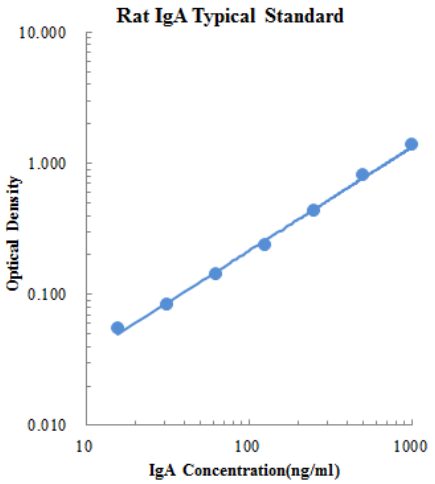
12) Add 100 µl of Stop Solution to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

13) 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	0.026	0.025	0.026	
15.63	0.058	0.056	0.057	0.032
31.25	0.091	0.091	0.091	0.066
62.50	0.162	0.154	0.158	0.133
125.00	0.291	0.285	0.288	0.263
250.00	0.531	0.504	0.518	0.492
500.00	0.944	0.948	0.946	0.921
1000.00	1.555	1.556	1.556	1.530



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