Technical Manual

Rat Estrogen ELISA Kit (RTFI01467)

- Catalogue Code: RTFI01467
- Competitive ELISA Kit
- Research Use Only

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1. Key features and Sample Types

Aliases:

ESRRG, Estrogen-related receptor gamma, ERR3, NR3B3, ERR gamma-2, ERRG2, ERRgamma, Estrogen receptor-related protein 3, NR3B3, Nuclear receptor subfamily 3 group B member 3, KIAA0832

Uniprot:

Detection method:

Competitive, Double Antibody

Sample Type:

Serum, Plasma and other biological fluids

Reactivity:

Rat

Range:

15.625-1000pg/ml

Sensitivity:

< 9.375pg/ml

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit at 4°C. Date of expiration will be on the ELISA Box label.

3. Description and Principle

The Assay Genie ELISA (enzyme-linked immunosorbent assays) assay kits are designed for the measurement of analytes in a wide variety of samples. As today‘s scientists demand high quality consistent data, we have developed a range of sensitive, fast and reliable ELISA kit assays to meet and exceed those demands.

This kit is based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with analyte of interest. During the reaction, the analyte in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to analyte. Excess conjugate and unbound sample or standard are washed from the plate, and HRP-Streptavidin (SABC) is added to each microplate well and incubated. TMB substrate solution is then added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

4. Kit Contents

Each kit contains reagents for either 48 or 96 assays including:

No.	Component	48-Well Kit	96-Well Kit	Storage
1	ELISA Microplate (dismountable)	8 x 6	8 x 12	2-8°C/-20°C
2	Lyophilized Standard	1 vial	2 vials	2-8°C/-20°C
3	Sample Dilution Buffer	10 mL	20 mL	2-8°C
4	Biotin-labelled Antibody (Concentrated)	30 uL	60 uL	2-8°C (Avoid Direct Light)
5	Antibody Dilution Buffer	5 mL	10 mL	2-8°C
6	HRP-Streptavidin Conjugate (SABC)	60 uL	120 uL	2-8°C (Avoid Direct Light)
7	SABC Dilution Buffer	5 mL	10 mL	2-8°C
8	TMB Substrate	5 mL	10 mL	2-8°C (Avoid direct light)
9	Stop Solution	5ml	10ml	2-8°C
10	Wash Buffer (25x)	15 mL	30 mL	2-8°C
11	Plate Sealer	3 pieces	5 pieces	
12	Manual	1	1	

Additional materials required:

1. 37°C incubator
2. Plate shaker
3. Plate Reader with 450nm filter
4. Precision pipettes and disposable pipette tips
5. Distilled water
6. Disposable tubes for sample dilution
7. Absorbent paper

Precautions:

1. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.

2. Ensure unopened and unused plate is kept dry to avoid contamination.

3. Before using the kit, centrifuge tubes to spin down standard & antibodies.

4. Avoid light for storage of TMB reagents.

5. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.

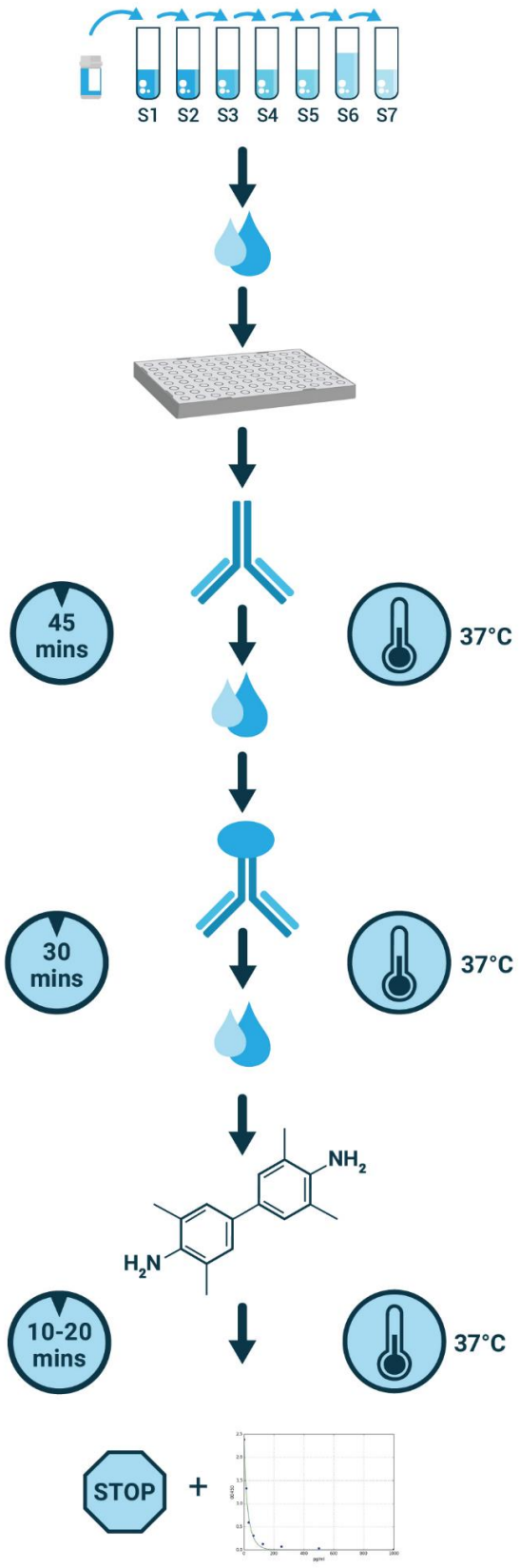
6. Duplicate wells are recommended for both standard and sample testing.

7. Do not let the microplate wells dry during assay. Dry plates will inactivate active components.

8. Do not reuse tips and tubes to avoid cross contamination.

9. Avoid using the reagents from different batches together.

5. Workflow Overview



Prepare plate standards and reagents

Wash plate x 2

Add 50µl of samples, standards and controls. Immediately add 50µl Biotin-labelled Antibody working solution.

Wash plate X3

Add 100µl SABC working solution.

Wash plate X5

Add 90µl TMB Substrate.

Add 50µl STOP solution and read absorbance @ 450nm

6. Sample Preparation

General considerations: According to best practices, extract protein & perform the experiment as soon as possible after sample collection. Alternatively, store the extracts at the designated temperature (-20°C/-80°C). For optimal results, avoid repeated freeze-thaw cycles.

Serum: If using serum separator tubes, allow samples to clot for 30 minutes at room temperature. Centrifuge for 20 minutes at 1,000x g. Collect the serum fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

If serum separator tubes are not being used, allow samples to clot overnight at 2-8°C. Centrifuge for 20 minutes at 1,000x g. Remove serum and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples at 4°C for 15 mins at 1000 × g within 30 mins of collection. Collect the plasma fraction and assay promptly or aliquot and store the samples at -80°C. Avoid multiple freeze-thaw cycles.

Note: Over haemolyzed samples are not suitable for use with this kit.

Cell culture supernatant: Collect the cell culture media by pipette, followed by centrifugation at 4°C for 20 mins at 1000 x g. Collect the clear supernatant and assay immediately.

Cell lysates: Commercial RIPA kits are recommended for preparation of cell lysate samples. For 2 x 10^6 cells, lyse cells in 0.5 mL RIPA buffer, removing DNA content. Determine the protein concentration using a BCA assay kit (BN01031) with each sample concentration being ≤ 300μg for analysis.

Tissue Homogenates: Rinse tissue with 1X PBS to remove excess blood. Mince tissue after weighing it and homogenize in PBS (the volume depends on the weight of the tissue. 9mL PBS (including protease inhibitors) would be appropriate for 1 gram of tissue. To further lyse cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 x g and the supernatant is removed for assaying. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Notes

Samples stored at 2-8°C should be used within 5 days, samples stored at -20°C should be assayed within 1 month and samples stored at -80°C should be assayed within 2 months to reduce the loss of bioactivity. Avoid multiple freeze-thaw cycles. Hemolyzed samples are not suitable for this assay.

7. Standard and Reagent Preparation

Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350μl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

Automated Washing

Aspirate all wells, then wash plate with 350μl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute (Note: set the height of the needles; be sure the fluid can be taken up completely).

Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

Note: Matrix components in the sample will affect test results, samples need to be diluted at least ½ with Sample Dilution Buffer before testing.

Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use.

1. Wash Buffer:

Dilute 30ml (15 ml for 48 well assay) of Concentrated Wash Buffer into 750 ml (375 ml for 48 well assay) of Wash Buffer with deionized or distilled water. Store unused solution at 4°C. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

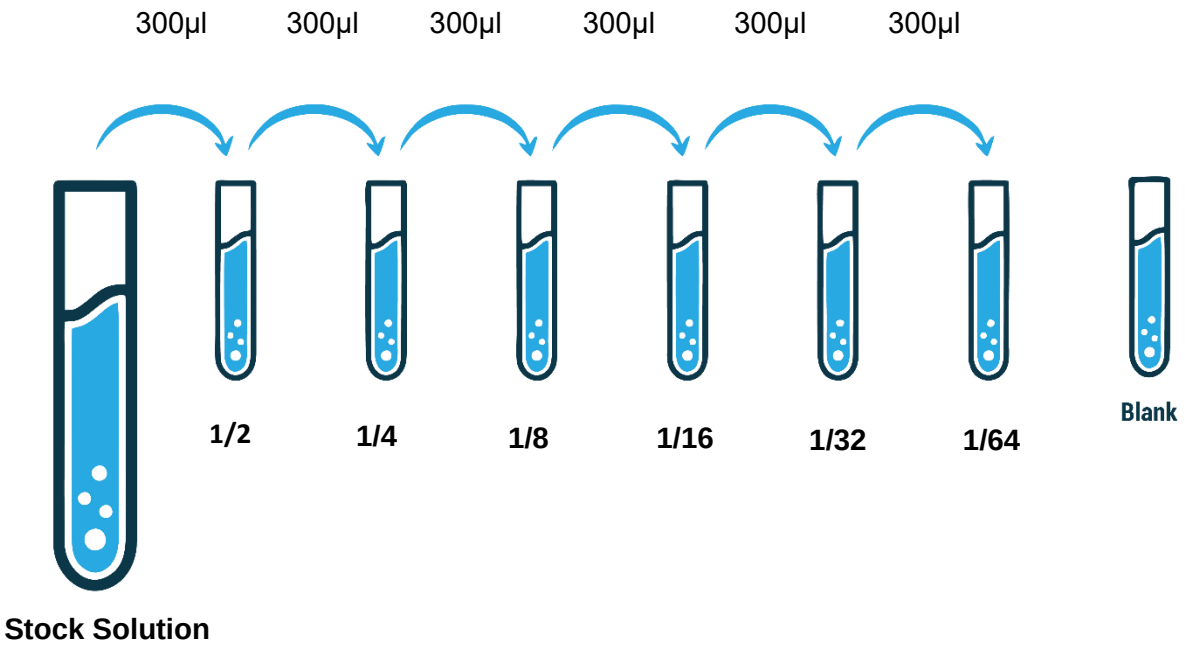
2. Standard Dilution:

1). Add 1ml of Sample dilution buffer into one Standard tube (labelled as Stock Solution), keep the tube at room temperature for 10 min and mix thoroughly.

Note: If the standard vial concentration is different to the highest value in the range (please see page 3), please dilute using sample buffer to match highest range value to create stock solution.

2). Label 7 Eppendorf tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Aliquot 300µl of the Sample dilution buffer into each tube. Add 300µl of the above (Stock Solution) standard solution into 1st tube and mix thoroughly. Transfer 300µl from 1st tube to 2nd tube and mix thoroughly. Transfer 300µl from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample dilution buffer is used as blank control.

DILUTION SERIES



Note: The standard solutions are best used within 2 hours. The standard solution series should be kept at 4°C for up to 12 hours. Or store at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Preparation of biotin-labeled Antibody working solution:

Prepare within 1 hour of starting the experiment.

1. Calculate the total volume of the working solution: 50μl / well × quantity of wells (Allow 100-200μl more than the total volume).

2. Dilute the biotin-detection Antibody with antibody dilution buffer at 1:100 and mix thoroughly (i.e. Add 1μl of biotin-labeled Antibody into 99μl of Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) working solution:

Prepare within 30 minutes of starting the experiment.

1. Calculate the total volume of the working solution: 100μl / well × quantity of wells. (Allow 100-200μl more than the total volume)

2. Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC dilution buffer.)

8. Assay Procedure

Equilibrate the TMB substrate for at least 30 min at 37°C before use. When diluting samples and reagents, they must be mixed completely and evenly. It is recommended to plot a standard curve for each test.

1. **Set standard, test sample and control (blank) wells** on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate**.** **Wash plate 2 times before adding standard, sample and control (blank) wells.**

2. **Add Sample and Biotin-labeled Antibody:** Add 50μL of Standard, Blank or Sample per well. The blank well is added with Sample Dilution Buffer. Immediately add 50 μL of biotin-labeled Antibody working solution to each well. Cover with the plate sealer provided. Gently tap the plate to ensure thorough mixing. Incubate for 45 minutes at 37°C. (Solutions are added to the bottom of micro-ELISA plate well, avoid touching plate walls and foaming).

3. **Wash:** Aspirate each well and wash, repeating the process X3 according to instructions. **Let the wash buffer stay in the wells for 1-2 minutes each time.** After final wash, remove any remaining wash buffer by aspirating or decanting

4. **HRP-Streptavidin Conjugate (SABC):** Add 100μL of SABC working solution to each well.

5. **Incubate:** Cover with a new Plate sealer. Incubate for 30 minutes at 37°C.

6. **Wash:** Repeat the aspiration/wash process X5 according to instructions. **Let the wash buffer stay in the wells for 1-2 minutes each time.**

7. **TMB Substrate:** Add 90μL of TMB Substrate to each well. Cover with a new plate sealer. Incubate for about 10-20 minutes in the dark at 37°C. Protect from light.

   Note: The reaction time can be shortened or extended according to the actual colour change, but not more than 30 minutes. When apparent gradient appears in standard wells, you can terminate the reaction.

8. **Stop:** Add 50μL of Stop Solution to each well. Wells will turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.

9. **OD Measurement:** Determine the optical density (OD Value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters.

9. Data Analysis

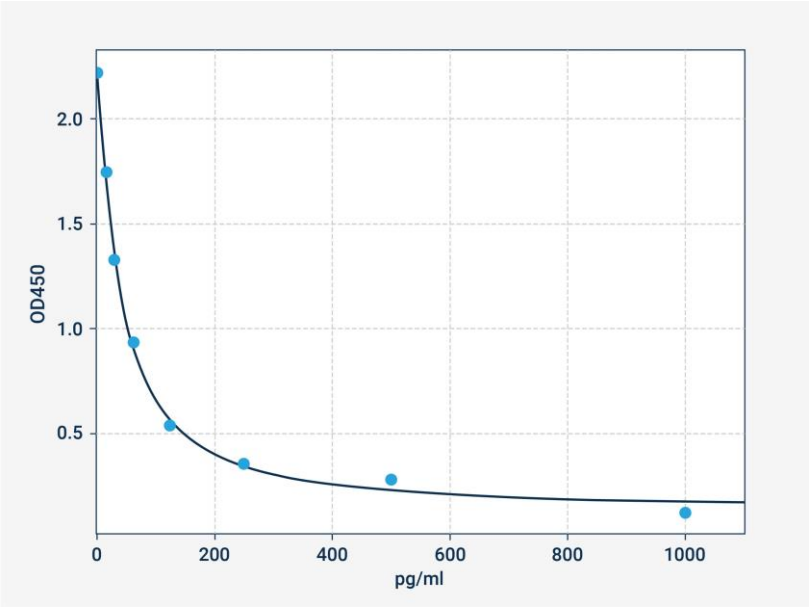
Regarding calculation, the standard curve can be plotted as the O.D.450 of each standard solution (Y) vs, the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. Curve Expert 1.3 or 1.4 is recommended for data analysis.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

10. Typical Data & Standard Curve

Standard Curve

Results of a typical standard run of a ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



Specificity

This assay has high sensitivity and excellent specificity for detection of . No significant cross-reactivity or interference between and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between and all the analogues, therefore, cross reaction may still exist.

Recovery

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

Not Available

Linearity

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of and their serial dilutions.

Not Available

Precision

- **Intra-Assay:** CV<8%
- **Inter-Assay:** CV<10%

Stability

The stability of the ELISA Kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage conditions.

Standard (n=5)	**37°C for 1 month**	**4°C for 6 months**
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end**.**

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