

Mouse Endothelin 2 (ET-2) ELISA Kit

Cat #: orb2809615 (manual)

Size: 96 tests / 48 tests

For research use only, not for clinical diagnosis.

ET-2 Introduction

Endothelin-2 (ET-2) is a protein encoded by the ET-2 gene. It belongs to a family of three endothelin peptide isoforms (ET-1, ET-2, ET-3) that can cause blood vessel constriction. It is encoded by genes on different chromosomes, primarily produced in the vascular endothelial cells of the kidneys, placenta, uterus, heart, central nervous system, and intestines. It exerts its effects by binding to two different G protein-coupled receptors (GPCRs), namely the endothelin A receptor (EDNRA) and the endothelin B receptor (EDNRB).

Detection Principle

This kit uses the enzyme-linked immunosorbent assay (ELISA) competitive method. Mouse Endothelin-2 (ET-2) antigen is pre-coated in the wells, and then samples, standards, biotin-labeled antibodies, and HRP enzyme conjugate are added sequentially. After incubation and washing, the substrate TMB is added for color development. TMB is converted to blue in the presence of horseradish peroxidase (HRP) and then converted to the final yellow color under acidic conditions. The intensity of the color is negatively correlated with the concentration of mouse Endothelin-2 (ET-2) in the sample. The absorbance (OD value) is measured at 450nm using an enzyme-labeled instrument to calculate the sample concentration.

Detection Range: 7.81-500pg/mL

Sensitivity: 3.87pg/mL

Specificity: detects mouse ET-2 in samples with no significant cross-reactivity with its analogues.

Product Composition

| Reagents | Specifications (96T) | Specifications (48T) | Storage Conditions |
|-------------------------|----------------------|----------------------|--------------------|
| Pre-coated Assay Plate | 8×12 | 8×6 | 2-8°C |
| Standard | 2 tubes | 1 tube | 2-8°C |
| Universal Diluent | 2×20 mL | 1×20 mL | 2-8°C |
| Biotin-antibody (100×) | 60 µl | 30 µl | 2-8°C |
| Streptavidin-HRP (100×) | 120 µl | 60 µl | 2-8°C |
| Wash Buffer (20×) | 2×10 mL | 1×10 mL | 2-8°C |

| | | | |
|---------------|----------|----------|-------|
| TMB Substrate | 10 ml | 5 ml | 2-8°C |
| Stop Solution | 6 ml | 3 ml | 2-8°C |
| Plate Sealer | 4 pieces | 4 pieces | |
| Manual | 1 copy | 1 copy | |

Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision pipette with disposable tips: 0.5-10uL, 5-50uL, 20-200uL, 200-1000uL
3. 37°C incubator
4. Deionized or distilled water

Precautions

1. Incubation is carried out in strict accordance with the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Refrigerate reagents immediately after use.
2. Incorrect plate washing may result in inaccurate results. Make sure to suction out as much liquid as possible from the wells before adding the substrate. Do not allow the micropores to dry out during the incubation process.
3. Eliminate residual liquid and finger marks from the bottom of the plate, otherwise the OD value will be affected.
4. The substrate chromogenic solution should be colorless or very light in color, and the substrate solution that has turned blue cannot be used.
5. Avoid cross-contamination of reagents and specimens to avoid false results.
6. Avoid direct exposure to strong light during storage and incubation.
7. No reaction reagent should come into contact with the bleaching solvent or the strong gases emitted by the bleaching solvent. Any bleaching component will destroy the biological activity of the reaction reagent in the kit.
8. Expired products cannot be used, and components with different article numbers and batch numbers should not be mixed.
9. Recombinant proteins from sources other than the kit may not match the antibody of this kit and may not be recognized.
10. If there is a possibility of disease transmission, all samples should be managed, and samples and testing devices should be handled in accordance with the prescribed procedures.

Sample Collection and Requirements

- 1. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample**, and it is recommended to estimate the concentration of the analyte in the sample through relevant literature before the experiment and determine the actual concentration of the sample through the pre-experiment Condition. If the concentration of analyte in the sample is too high or too low, dilute or concentrate the sample appropriately.
- If the sample to be tested is not among the samples listed in the manual, it is recommended to do a pre-test to verify the validity of the test.
- 3. Serum:** Whole blood specimens collected in serum separators are stored at room temperature for 2 hours or 4°C overnight, then centrifuge at 1000×g for 20 minutes, and the supernatant can be taken or stored at -20°C or -80°C, but repeated freeze-thaw should be avoided.
- 4. Plasma:** Collect specimens with EDTA or heparin as anticoagulants and centrifuge the specimen at 1000×g at 2-8°C for 15 minutes within 30 minutes of collection, take the supernatant for detection, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.
- 5. Tissue homogenate:** Rinse the tissue with pre-chilled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate can affect the measurement), and mince the tissue after weighing. The minced tissue is compared to the corresponding volume of PBS (generally according to the weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9mL of PBS, the specific volume can be adjusted appropriately according to the needs of the experiment, and a record should be made. It is recommended to add protease inhibitors to PBS) to a glass homogenizer and grind well on ice. To further lyse the histiocytes, the homogenate can be sonicated, or repeatedly freeze-thaw. Finally, the homogenate was centrifuged at 5000×g for 5~10 minutes, and the supernatant was taken for detection.
- 6. Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes to detect, or store the supernatant at -20°C or -80°C, but avoid repeated freeze-thaw cycles.
- 7. Cell lysis buffer:** Adherent cells are gently washed with pre-cooled PBS, then digested with trypsin, and centrifuged at 1000×g for 5 minutes to collect the cells; suspended cells can be directly centrifuged for collection. The collected cells are washed with pre-cooled PBS three times, and 150-200μL of PBS is added for resuspension per 1×10^6 cells (it is recommended to add protease inhibitors to the PBS, if the content is very low, the volume of PBS can be appropriately reduced) and the cells are disrupted through repeated freeze-thaw cycles or ultrasound. The extract is centrifuged at 1500×g for 10 minutes at 2-8°C, and the supernatant is collected for detection.
- 8. Other biological specimens:** Centrifuge at 1000×g for 20 minutes, and take the supernatant for detection.
- 9. Appearance of the sample:** The sample should be clear and transparent, and the suspended solids should be centrifuged and removed.
- 10. Sample preservation:** If the sample is tested within 1 week after collection, it can be stored at 4°C, if it cannot be tested in time, please divide it into one-time use and freeze it at -20°C (test within 1 month), or -80°C (testing within 6 months), avoid repeated freezing and thawing, hemolysis of the specimen will affect the final test result, so hemolyzed specimens should not be tested for this test.

Sample Dilution Protocol

Please estimate the concentration range of the samples in advance. If your test sample needs to be diluted, refer to the dilution protocols below.

Dilution 100 times: One-step dilution. Take 5 μ L of the sample and add it to 495 μ L of universal diluent to make a 100-fold dilution;

Dilution 1000 times: Two-step dilution. Take 5 μ L of the sample and add it to 95 μ L of universal diluent to make a 20-fold dilution, then take 5 μ L of the 20-fold diluted sample and add it to 245 μ L of universal diluent to make a 50-fold dilution, resulting in a total dilution of 1000 times;

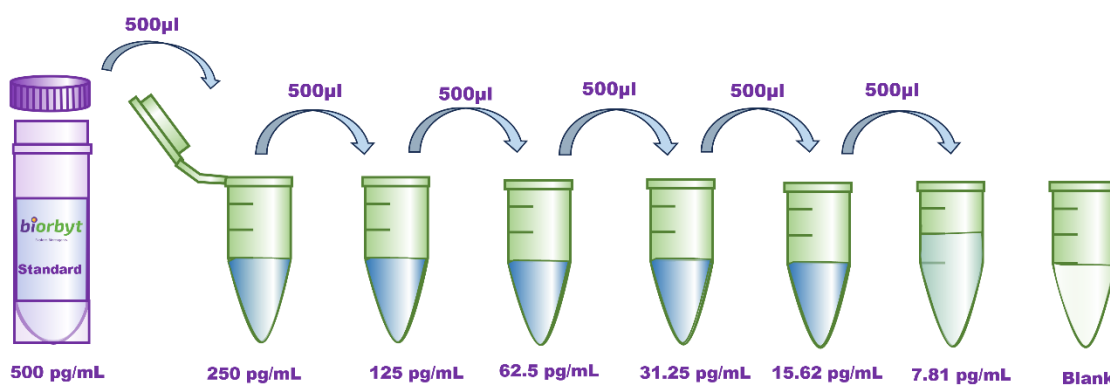
Dilution 100000 times: Three-step dilution. Take 5 μ L of the sample and add it to 195 μ L of universal diluent to make a 40-fold dilution, then take 5 μ L of the 40-fold diluted sample and add it to 245 μ L of universal diluent to make a 50-fold dilution, and finally take 5 μ L of the 2000-fold diluted sample and add it to 245 μ L of universal diluent to make a 50-fold dilution, resulting in a total dilution of 100000 times;

For each dilution step, the volume taken should be no less than 3 μ L, and the dilution factor should not exceed 100 times. Each dilution step must be mixed thoroughly to avoid foaming.

Reagent Preparation

1. Remove the kit from the refrigerator 10 minutes in advance and equilibrate to room temperature.
2. **Standard gradient working solution:** Add 1mL of universal dilution to the lyophilized standard, let it stand for 15 minutes, let it completely dissolve, and then mix gently (at a concentration of 500 pg/mL), then dilute at the following concentrations: 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.25 pg/mL, 15.62 pg/mL, 7.81 pg/mL, 0 pg/mL.

Doubling dilution method: take 7 EP tubes, add 500 μ L of general dilution solution to each tube, and draw 500 μ L from 500pg/mL standard working solution to the first EP tube to mix well to prepare 250pg/mL standard working solution, and then pipette and mix well according to this step. The last tube is used directly as a blank well, and there is no need to draw liquid from the penultimate tube, as shown in the figure below.



3. **Preparation of biotinylated antibody detection solution:** 15 minutes before use, centrifuge the concentrated biotinylated antibody at 1000 \times g for 1 minute, and dilute the 100 \times concentrated biotinylated antibody into a 1 \times working concentration (10 μ L concentrated + 990 μ L universal diluent) in a universal diluent, and use it on the same day.

4. Preparation of enzyme conjugate working solution: Centrifuge 100× concentrated streptavidin-HRP at 1000×g for 1 minute before use, and dilute 100× concentrated streptavidin-HRP to 1× working concentration in a universal diluent (10 µL concentrated + 990 µL universal diluent) for use on the same day.

5. Preparation of 1× washing solution: take 10ml of 20× washing solution to 190ml of distilled water (the concentrated washing solution taken out of the refrigerator may have crystallization, which is a normal phenomenon, it can be placed at room temperature, shake evenly, and then configure after the crystallization is completely dissolved).

Operation Steps

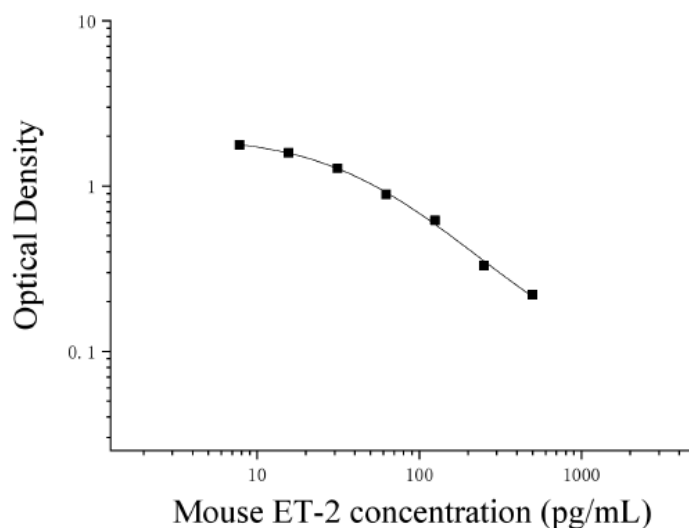
1. Remove the desired slats from the foil pouch after 10 minutes of room temperature equilibration, and place the remaining slats back at 4°C with a ziplock bag.
2. Loading: Add 50 µl of samples or standards of different concentrations to the corresponding wells, and add 50 µL of universal diluent to the blank wells, immediately add 50µL of Biotin-antibody working solution to each well, incubate for 1 h at 37°C after covering with the plate sealer. (Suggestion: Samples to be tested are diluted by a minimum of 1-fold with a universal diluent before being added to the microplate for testing to reduce the impact of matrix effects on the test results, and the sample concentration should be multiplied by the corresponding dilution factor when calculating the sample concentration. All samples and standards to be tested are recommended to be double-well).
3. Wash the plate: Discard the liquid, add 300µL of 1x wash solution to each well, let it stand for 1 minute, shake off the wash solution, pat dry on absorbent paper, and repeat the wash 3 times (you can also use a plate washer to wash the plate).
4. Add streptavidin-HRP working solution: Add 100µL of streptavidin-HRP working solution to each well, cover the plate and incubate at 37°C for 30 minutes.
5. Wash the plate: Discard the liquid and wash the plate 5 times according to the washing method in step 3.
6. Add TMB Substrate: 90 µL of TMB Substrate was added to each well, covered with a plate sealer, and incubated at 37°C for 15 minutes in the dark.
7. Add stop solution: Remove the microplate, add 50 µL of stop solution directly to each well, and immediately measure the OD value of each well at a wavelength of 450 nm.

Result Judgment

1. Calculate the average OD value of the standard and sample replicate wells and subtract the OD value of the blank well as the correction value. Using the concentration as the abscissa and the OD value as the ordinate, the standard curve of the four-parameter logistic function is drawn on a double-logarithmic coordinate paper (the value of the blank group is removed when plotting).
2. If the OD value of the sample is higher than the upper limit of the standard curve, it should be diluted appropriately and remeasured and multiplied by the corresponding dilution factor when calculating the sample concentration.

The following data and standard curves are for reference only, and experimenters need to establish standard curves according to their own experiments.

| | | | | | | | | |
|-----------------------|------|------|------|------|-------|-------|------|------|
| Concentration (pg/mL) | 500 | 250 | 125 | 62.5 | 31.25 | 15.62 | 7.81 | 0 |
| OD value | 0.22 | 0.33 | 0.62 | 0.89 | 1.28 | 1.59 | 1.78 | 2.12 |



Note: This figure is for reference only, and the specimen content should be calculated using the standard curve drawn for the same test standard.

Kit Performance

1. Repeatability: The coefficient of variation within the plate is less than 10%, and the coefficient of variation between plates is less than 10%.
2. Recovery: Recovery was calculated by adding 3 different concentration levels of mouse ET-2 to selected healthy mouse serum, plasma, and cell culture supernatants.

| Sample type | Range | Average recovery |
|--------------------------------|--------|------------------|
| Serum (n=8) | 82-105 | 96 |
| Plasma (n=8) | 87-98 | 93 |
| Cell culture supernatant (n=8) | 83-114 | 96 |

3. Linear dilution: Linearity was assessed by adding high concentrations of mouse ET-2 to 4 selected healthy mouse serum, plasma, and cell culture supernatants, respectively, and diluting within the standard curve kinetics.

| Dilution range | Recovery (%) | Serum | Plasma | Cell culture supernatant |
|----------------|----------------------|--------|--------|--------------------------|
| 1: 2 | Range (%) | 83-113 | 88-106 | 83-94 |
| | Average recovery (%) | 99 | 94 | 86 |
| 1: 4 | Range (%) | 90-111 | 86-99 | 83-102 |
| | Average recovery (%) | 103 | 90 | 91 |

Troubleshooting

If the experimental results are not good, please take photos of the color development results in a timely manner, save the experimental data, keep the used plates and any unused reagents, and then contact our technical support for assistance. You may also refer to the following materials.

| Problems | Possible Causes | Solutions |
|--|--|---|
| Linear deviation of the standard curve | Standard sample dilution is incorrect. | Ensure that the standard sample is dissolved and diluted according to the recommended method. |
| | Inaccurate pipetting. | Regularly calibrate the pipette and check the sealing of the tips. |
| | Evaporation of reaction solution. | Seal the enzyme labelling plate with sealing film. |
| | Incomplete washing of the plate. | Sufficient washing cycles and adequate washing liquid should be added. |
| | Impurities at the bottom of the wells. | Clean the bottom of the plate before reading. |
| Weak signal or No signal | Insufficient incubation time. | Ensure sufficient incubation time. |
| | Incubation temperature is incorrect. | Incubate at the recommended temperature. |
| | Insufficient reagent volume added. | Check the pipette and follow the operational steps rigorously. |
| | Incorrect dilution. | Check the reagent dilution steps. |
| | Enzyme conjugate inactivation. | Mix the enzyme conjugate and substrate, and check through the colour reaction. |
| Low OD | The microplate reader is set up incorrectly. | Check the instrument's wavelength. |
| | The stopping solution was not added. | Add an appropriate amount of stopping solution. |

| | | |
|-----------------|---|--|
| | The waiting time before reading the plate is too long. | Read the plate in a timely manner. |
| | The sample concentration is too high. | Determine the appropriate dilution factor through preliminary experiments. |
| | The sample concentration is too low. | Determine the appropriate dilution factor through preliminary experiments. |
| High background | The developing solution is contaminated. | Replace the developing solution. |
| | The developing time is too long. | Control the developing time. |
| | Incorrect dilution of the antibody or enzyme conjugate. | Use the recommended dilution method. |
| | Washing of the plates is incomplete. | Ensure enough washing cycles and add sufficient washing solution. |