Rat C-reactive protein (CRP) ELISA research kit,

Product Code: 248433

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Product Introduction

This kit uses a double-antibody sandwich method. A rat C-reactive protein (CRP) capture antibody is pre-coated on the microplate to bind the target in standards and samples. A biotin-labeled antibody then binds to the target, followed by an enzyme conjugate that binds to the biotin-labeled antibody to form an immune complex.

After adding TMB color development solution, wells containing the target turn blue. After stop solution is added, the color turns yellow. Free components are removed during washing. Read absorbance at 450 nm with a microplate reader to determine the OD value. Color intensity is proportional to the rat CRP concentration, and the sample concentration is calculated from the standard curve.

- Detection range: 0.14-9 ng/mL
- Sensitivity: 0.068 ng/mL
- Specificity: Detects rat CRP with no significant cross-reactivity with other analogs
- Repeatability: Within-plate and between-plate coefficients of variation are both <10%

Package Contents

Item No.	Component	48T	96T
248433.1	Pre-coated microplate	8 wells × 6 strips	8 wells × 12 strips
248433.2	Standard	1 vial	2 vials
248433.3	Standard & Sample Diluent	10 mL	15 mL
248433.4	Biotin-labeled antibody (100×)	1 vial	1 vial
248433.5	Biotin-labeled antibody diluent	10 mL	15 mL
248433.6	Concentrated enzyme conjugate (100×)	1 vial	1 vial
248433.7	Enzyme Conjugate Diluent	10 mL	15 mL
248433.8	TMB Color Development Solution A	3 mL	6 mL
248433.9	TMB Color Development Solution B	3 mL	6 mL
248433.10	Stop Solution	3 mL	6 mL
248433.11	Wash Solution (20×)	10 mL × 1	10 mL × 2
248433.12	Plate Sealer Film	2 sheets	4 sheets
248433.m	Manual	1 copy	1 copy

Quality Standards and Safety Instructions

Raw Material and Packaging Name	Quality Standard	Main Toxicity
Pre-coated Microplate	—	—
Standard	—	—
Standard & Sample Diluent	—	—
Biotin-Labeled Antibody (100×)	—	—
Biotin-Labeled Antibody Diluent	—	—
Concentrated Enzyme Conjugate (100×)	—	—
Enzyme Conjugate Diluent	—	—
TMB Color Development Solution A	—	—
TMB Color Development Solution B	—	—
Stop Solution	—	—
Wash Solution (20×)	—	—

Sealing film	—	—

Transportation and Storage

Transport this product with ice packs.

Store at 2-8°C. Shelf life: 180 days.

Sample Handling

> The kit detection range is not the same as the analyte concentration range in the sample. Estimate the expected analyte concentration from relevant literature before the experiment, then confirm the actual concentration with a preliminary test. If the analyte concentration is too high or too low, dilute or concentrate the sample appropriately.
>
> If the sample type is not listed below, perform a preliminary experiment to verify detection validity.

Sample Types and Preparation

1. **Serum:** Let whole blood collected in serum separation tubes stand at room temperature for 2 hours or at 4°C overnight. Centrifuge at 1000×g for 20 minutes and collect the supernatant. Store at -20°C or -80°C if needed. Avoid repeated freeze-thaw cycles.

2. **Plasma:** Use EDTA or heparin as the anticoagulant. Within 30 minutes after collection, centrifuge at 2-8°C and 1000×g for 15 minutes. Collect the supernatant for testing, or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

3. **Tissue homogenate:** Rinse tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood. Weigh and mince the tissue, then mix with PBS at a general weight-to-volume ratio of 1:9. For example, 1 g tissue corresponds to 9 mL PBS. The volume may be adjusted as needed, but records should be kept. Adding protease inhibitors to PBS is recommended. Homogenize thoroughly on ice using a glass homogenizer. To further lyse tissue cells, use sonication or repeated freeze-thaw cycles. Finally, centrifuge at 5000×g for 5-10 minutes and collect the supernatant for testing.

4. **Cell culture supernatant:** Centrifuge at 1000×g for 20 minutes and collect the supernatant for testing, or store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

5. **Other biological samples:** Centrifuge at 1000×g for 20 minutes and collect the supernatant for testing.

6. **Cell lysate:** For adherent cells, wash with pre-cooled PBS, digest with trypsin, and centrifuge at 1000×g for 5 minutes to collect the cells. For suspension cells, collect directly by centrifugation. Wash collected cells with pre-cooled PBS 3 times. For every 1 × 10^6 cells, add 150-200 µL PBS to resuspend. Adding protease inhibitors in advance is recommended. Disrupt cells by repeated freeze-thaw cycles or sonication. Finally, centrifuge at 2-8°C and 1500×g for 10 minutes, then collect the supernatant for testing.

7. **Sample appearance:** Samples should be clear and transparent. Remove suspended matter by centrifugation.

8. **Sample storage:** If testing will be performed within 1 week after collection, samples may be stored at 4°C. If testing cannot be performed promptly, aliquot for single use and store at -20°C for up to 1 month or at -80°C for up to 6 months. Avoid repeated freeze-thaw cycles. Hemolyzed samples should not be used because hemolysis affects the final test result.

Procedure

1. Remove the kit from the refrigerator 10 minutes in advance and allow it to equilibrate to room temperature.

2. **Preparation of the standard gradient working solution:** Add 1 mL standard & sample diluent to the standard vial to dissolve it. Cap and let stand at room temperature for about 10 minutes. Prepare seven 1.5 mL centrifuge tubes labeled S6, S5, S4, S3, S2, S1, and S0. Add 250 µL standard & sample diluent to each tube. From S7, transfer 250 µL standard into S6 and mix gently by

pipetting up and down. Transfer 250 µL from S6 to S5 and mix gently. Continue the serial 2-fold dilution in the same way. The resulting concentrations are 9 ng/mL, 4.5 ng/mL, 2.25 ng/mL, 1.12 ng/mL, 0.56 ng/mL, 0.28 ng/mL, and 0.14 ng/mL. S0 contains standard & sample diluent only and is 0 ng/mL.

3. **Preparation of the biotin-labeled antibody working solution:** Fifteen minutes before use, centrifuge the 100× concentrated biotin-labeled antibody at 1000×g for 1 minute. Dilute it with biotin-labeled antibody diluent to a 1× working concentration. Example: 10 µL concentrate + 990 µL diluent. Use the working solution on the same day.

4. **Preparation of the enzyme conjugate working solution:** Fifteen minutes before use, centrifuge the 100× concentrated enzyme conjugate at 1000×g for 1 minute. Dilute it to a 1× working concentration. Example: 10 µL concentrate + 990 µL diluent. Use the working solution on the same day.

5. **Preparation of 1× wash solution:** Dilute the 20× wash solution with distilled water at 1:20. For example, add 1 mL of 20× wash solution to 19 mL distilled water. Crystals may appear in refrigerated concentrated wash solution; this is normal. Allow it to reach room temperature, mix gently, and wait until the crystals are fully dissolved before dilution.

6. **Preparation of TMB color development solution:** Ten minutes before use, mix TMB Color Development Solution A and Solution B at a 1:1 ratio. Protect from light.

Result Interpretation

1. Calculate the mean OD value for duplicate standards and samples. Use concentration as the x-axis and OD value as the y-axis, and plot the standard curve using a four-parameter logistic function on log-log graph paper. Exclude the blank group values when plotting.

2. If the sample OD value is below the upper limit of the standard curve, dilute appropriately, retest, and multiply the calculated concentration by the corresponding dilution factor.

Precautions

> This product is for scientific research use only and cannot be used for clinical diagnosis.

- Wear a lab coat, mask, and latex gloves during the experiment. Follow national biological laboratory safety regulations, especially when testing blood samples or other body fluids.
- For accurate results, use only the reagents provided and do not mix reagents from different batches.
- Results for the same analyte may differ when using kits from different sources or different detection methods.
- If the sample type is not listed in this manual, perform a preliminary test to verify detection validity.
- Avoid direct exposure to strong light during storage and incubation.
- If a chemical lysis buffer is used to prepare tissue homogenates or cell extracts, some chemicals may bias ELISA readings.
- Some recombinant proteins may not match the capture or detection antibodies in the kit and may fail to be detected.
- For reproducible results, strictly control each experimental step. Differences in sample collection, handling, and storage may also affect measured values.