Product Manual

Human Rheumatoid Factor IgM ELISA Kit

Catalog Number

PRB- 5066	96 assays
PRB- 5066	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Rheumatoid arthritis (RA) is a long-term autoimmune disorder that causes swollen and painful joints. Other parts of the body may also be affected, resulting in a low red blood cell count, inflammation around the lungs, and inflammation around the heart. Rheumatoid factor (RF) is the autoantibody (antibody directed against an organism's own tissues) that was first found in rheumatoid arthritis. RF is defined as an antibody that reacts against the Fc region of an IgG molecule (in other words an antibody against an antibody). Although the most common RF is of the isotype IgM, RF can exist as any other isotype of immunoglobulin including IgE, IgG, IgA, or IgD. In patients suspected of having any form of arthritis, the levels of RF are often quantified (even though positive results can be caused by other factors and negative results do not completely rule out RA). In tandem with the common signs and symptoms of RA, RF detection can help in both diagnosis and disease prognosis. The detection of RF in serum can also suggest an autoimmune response not related to rheumatoid arthritis like disorders associated with tissue or organ rejection. In these cases, RF may act as one of many serum markers for autoimmunity.

Cell Biolabs' Human Rheumatoid Factor IgM ELISA Kit is an immunoassay developed for the detection and quantitation of Rheumatoid Factor IgM in plasma, serum or other biological fluid samples. The kit detects RF IgM from human samples. Diluted patient samples, positive, and negative controls are added to a Goat IgG coated plate. The RF binds to the coated IgG and is then detected with a mouse monoclonal anti-Human RF IgM antibody, followed by an HRP conjugated secondary antibody. Each kit provides sufficient reagents to perform up to 96 assays including positive controls, negative controls, and unknown samples.

Related Products

1. PRB-5002: Pure-IP™ Western Blot Detection Kit
2. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
3. PRB-5034: Human Alpha 1 Antitrypsin ELISA Kit
4. PRB-5038: Human Beta 2 Microglobulin ELISA Kit
5. PRB-5039: Human Haptoglobin ELISA Kit
6. PRB-5041: Human Ceruloplasmin ELISA Kit
7. PRB-5044: Human Alpha 1 Acid Glycoprotein ELISA Kit
8. PRB-5049: Human Free PSA (f-PSA) ELISA Kit
9. PRB-5050: Human Cardiac Troponin I (cTnI) ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Goat IgG Antibody Coated Plate (Part No. 50661B): One strip well 96-well plate.
2. Anti-Human RF IgM Antibody (1000X) (Part No. 50662C): One 15 µL vial.
3. Secondary Antibody, HRP Conjugate (1000X) (Part No. 230003): One 20 µL vial.

4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. 10X RF Positive Plasma (Part No. 50663C): One 100 µL vial of Rheumatoid Factor Positive Human Plasma.
2. 10X RF Negative Plasma (Part No. 50664C): One 100 µL vial of Rheumatoid Factor Negative Human Plasma.

Materials Not Supplied

1. Plasma, Serum, or Other Biological Fluids
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the RF Positive Plasma and Negative Control Plasma at -20ºC and avoid multiple freeze/thaw cycles. Store the remainder of the kit at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-Human RF IgM Antibody (1000X) and Secondary Antibody, HRP Conjugate (1000X): Immediately before use dilute the Anti-Human RF IgM Antibody and the Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of 1X RF Positive and Negative Plasma Samples

Prepare 1X RF Positive or Negative Plasma Samples by diluting the 10X stock 1:10 into Assay Diluent or PBS containing 0.1% BSA. For example, for each control well, add 10 µL of the 10X RF Positive or Negative Plasma and 90 µL of Assay Diluent or PBS containing 0.1% BSA.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Dilute plasma samples as necessary with PBS containing 0.1% BSA immediately before running the ELISA. If running plasma samples undiluted, make sure the pH of the sample is near neutral. Plasma samples can be neutralized by adding 1:50 dilution of 1M Tris pH 7.0.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute serum samples as necessary with PBS containing 0.1% BSA immediately before running the ELISA. If running serum samples undiluted, make sure the pH of the sample is near neutral. Serum samples can be neutralized by adding 1:50 dilution of 1M Tris pH 7.0.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples as necessary with PBS containing 0.1% BSA immediately before running the ELISA. If running samples undiluted, make sure the pH of the sample is near neutral. Samples can be neutralized by adding 1:50 dilution of 1M Tris pH 7.0.

Assay Protocol

1. Prepare dilutions of plasma, serum, or other biological fluid samples in PBS containing 0.1% BSA as indicated in Preparation of Samples section above.
2. Add 100 µL of human RF IgM unknown sample, 1X RF Positive Plasma, or 1X RF Negative Plasma to the Goat IgG Antibody Coated Plate. Each unknown sample, control and blank should be assayed in duplicate.
3. Incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Anti-Human RF IgM Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
6. Wash the strip wells 3 times according to step 4 above.
7. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
8. Wash the strip wells 3 times according to step 4 above. Proceed immediately to the next step.

9. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

10. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
11. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figure demonstrates typical Rheumatoid Factor IgM ELISA Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

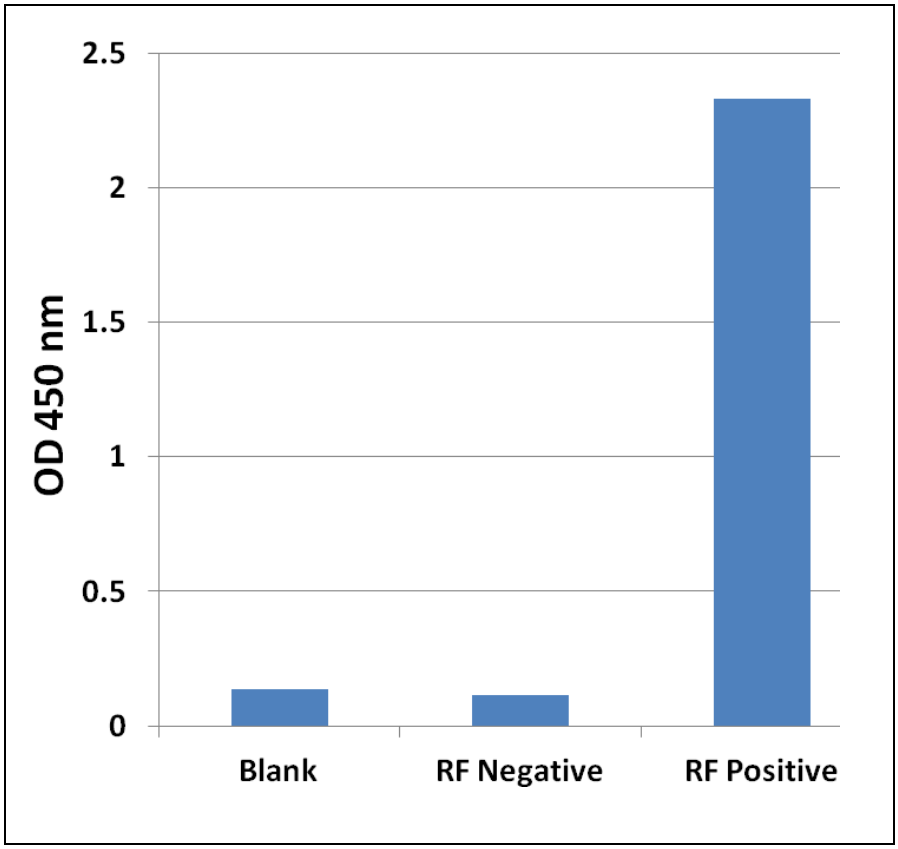

Figure 1: Rheumatoid Factor IgM Detection in Human Plasma. Wells were incubated in the absence of Plasma (Blank), in the presence of RF Negative Plasma (RF Negative), or in the presence of RF Positive Plasma (RF Positive) according to the Assay Protocol.

References

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