

Test Instructions

Enzyme Immunoassay for the Detection of IgM Rheumatoid Factors

Catalogue-No.: TC 60003

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Never mix reagents from different test kit lots.

1. Clinical Use

Rheumatoid factors are autoantibodies which are directed against the Fc-part of the IgG molecule. They are the most frequent autoantibodies in man.

Rheumatoid factors can belong to all classes of immunoglobulins. In practice importance has only been attached to rheumatoid factors of IgM type because only such rheumatoid factors can be detected with hitherto existing tests like latex agglutination or hemagglutination tests.

Rheumatoid factors of IgM type are found in about 75 % to 90 % of patients with rheumatoid arthritis depending on the sensitivity of the test. There exists a certain correlation between the presence of IgM-rheumatoid factors and the activity of the disease. But in about 10 % to 25 % of patients with rheumatoid arthritis rheumatoid factors are not detectable (seronegative rheumatoid arthritis), so that a negative test result does not exclude a manifest rheumatoid arthritis.

Apart from that in patients with a freshly established rheumatoid arthritis the synthesis of rheumatoid factors is retarded (for 4 weeks and more).

If rheumatoid factors are detectable in a patient this finding does not point to an existing rheumatoid arthritis because rheumatoid factors are also found in other diseases.

2. Principle of the Test

The test is based on the covalent immobilization of rabbit IgG to a chemically activated microtiter plate (patent pending) and subsequent binding of the rheumatoid factors from serum.

For the detection of rheumatoid factors bound in this way a peroxidase-labeled antibody is used which is directed against human IgM. After addition of a substrate solution, a color stain develops, the intensity of which is proportional to the concentration of the rheumatoid factors.

3. Material Provided

- coated microtiter strips (1 x 8), breakable	12 strips
- standards; ready to use,	1 vial each 750 µl per vial
1: 12.5 IU/ml	
2: 25 IU/ml	
3: 50 IU/ml	
4: 100 IU/ml	
5: 200 IU/ml (containing sodium azide)	
- negative control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- positive control serum, ready to use, (contains sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x), (contains thimerosal)	1 bottle 50 ml
- sample buffer concentrate (5x), (contains sodium azide)	1 bottle 22 ml
- conjugate buffer ready to use, (contains thimerosal)	1 bottle 20 ml
- peroxidase conjugate, anti-human IgM, concentrate (100x),	1 vial 200 µl
- peroxidase substrate, (TMB), ready to use,	1 bottle 12 ml
- stopping solution, H ₂ SO ₄ , ready to use,	1 bottle 12 ml

4. Preparation of Reagents

Allow the kit to reach room temperature!

4.1 Preparation of Washing Buffer

If any salt has been crystallized inside the bottle it must be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2 Preparation of Sample Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 unit sample buffer concentrate with 4 units distilled water. The ready to use buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of Standards

The standards are ready to use.

4.4 Preparation of Control Sera

The control sera are ready to use.

4.5 Preparation of Sera

Use serum or plasma samples freshly collected or freeze samples at - 20 °C. Do not use samples, that are repeatedly thawed and frozen. Do not use serum samples inactivated by heat treatment at 56 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1 : 100 with diluent buffer (10 µl sample to 1 ml buffer).

4.6 Preparation of Conjugate

The amount of conjugate dilution daily required, is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1 : 100 with dilution buffer (for 1 plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining solution should be disposed of.

4.7 Preparation of the Substrate

The TMB-substrate solution is ready to use. Used substrate bottle should be closed carefully. Store substrate solution at 4 - 8 °C protected from light.

4.8 Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 - 8 °C.

4.9 Stopping Solution

H₂SO₄ (caution!)

5. Test Procedure

- **Pipette 100 µl sample dilution** resp. (undiluted) standards and control sera into each well, for blanks use diluent buffer instead of sample dilution, seal wells with adhesive foil
- **Incubate for 1 hour** at room temperature (RT)
- **Rinse wells 3 x** with min. 200 µl washing buffer per well

- **Pipette 100 µl of conjugate dilution** into each well, seal wells with adhesive foil
- **Incubate for 30 minutes** at RT
- **Rinse wells 3 x** with min. 200 µl washing buffer per well
- **Pipette 100 µl substrate solution** into each well
- **Incubate for 10 minutes** at RT in the dark. At room temperatures above 25 °C the substrate incubation time could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µl stopping reagent** into each well
- **Measure at 450 nm** within the next 30 min after stopping

6. Interpretation of Results

Calibrate measured absorbances against concentrations/units of standards (12.5 IU/ml, 25 IU/ml, 50 IU/ml, 100 IU/ml, 200 IU/ml) in semilog. Determine the units of the examined sera from the standard curve directly.

Results above 15 IU/ml (cut off value) are considered positive.

To prove the functionality of the test, the determined value for the positive control serum is to be expected within the range labeled on the vial. The result of the negative control has to be lower than the cut off value of the test.

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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