

Test Instructions

Enzyme Immunoassay for the Detection of Anti-U1-snRNP Antibodies (cut off test)

Catalogue-No.: TC 70022

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Reagents from different test kit lots must not be mixed.

1. Clinical Use

Antinuclear antibodies are directed against various ubiquitous antigens in the cell nucleus. The detection of these antibodies plays an important role in the diagnosis of rheumatic diseases. In this regard, antibodies directed against proteins of the spliceosome complex U1-snRNP are of central diagnostic importance. The complex consists of a uridine (U)-rich RNA molecule and different proteins, such as the group of Sm proteins, U1-specific ribonucleoproteins A and C (RNP-A, -C), and the so-called 68 kD protein. Sm proteins also occur in other types of spliceosomal U-RNA (e.g., U2, U4/U6, and U5).

Until now, the detection of RNP autoantibodies was a diagnosis of exclusion. Conventional methods were able to prepare native Sm antigens, but were unable to isolate RNPs alone without Sm proteins. As a result, no definitive determination of the presence or absence of RNP antibodies could be made in patients with Sm-positive serum. Now, the IMTEC assay uses a balanced mixture of the three recombinant RNP proteins A, C and 68-kD, which are bound to the solid phase. Thus allowing the direct and unequivocal detection of RNP antibodies

U1-RNP antibodies are considered to be a diagnostic marker of mixed connective tissue disease (MCTD), which is also referred to as "Sharp's syndrome. Used in this indication, the antibodies achieve a sensitivity of 100% (per definition) and a specificity of 98% in the absence of both Sm and dsDNA antibodies.

2. Principle of the Test

The test is based on the immobilisation of recombinant U1-snRNP to a solid phase (polystyrene) and subsequent binding of the anti-U1-snRNP-antibodies. For the detection of antibodies bound in this way a peroxidase-labelled antibody is used which is directed against human IgG. After addition of a substrate solution, a color stain develops, the intensity of which is proportional to the concentration and/or the avidity of the antibodies.

3. Material Provided

- coated microtiter strips (1 x 8), breakable	12 strips
- cut off-control, ready to use, (contains sodium azide)	1 vial 2 ml
- 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,	1 bottle 20 ml
- peroxidase conjugate, anti-human IgG, concentrate (100x),	1 vial 200 µl
- peroxidase substrate solution, (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

Should any of the salts be crystallized they should 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

Dilute one unit sample buffer concentrate with 4 units distilled water. Should any of the salts be crystallized they should be resolved before use. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of the cut off-control

The cut off control sample is ready to use.

4.4 Preparation of Control Sera

The control sera are ready to use.

4.5 Preparation of Sera

Allow sera to reach room temperature (30 min). Dilute sera 1:100 with sample diluent buffer (10 µl serum with 1.0 ml buffer).

4.6 Preparation of Conjugate

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

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

1. **Pipette 100 µl serum dilution** or (undiluted) standards and control sera into each well, for blanks use ready to use dilution buffer instead of serum dilution, seal wells with adhesive foil
2. **Incubate for 1 hour** at room temperature (RT)
3. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well
4. **Pipette 100 µl of conjugate dilution** into each well, seal wells with adhesive foil
5. **Incubate for 30 minutes** at RT
6. **Rinse off the wells 3 x** with min. 200 µl washing buffer per well
7. **Pipette 100 µl substrate solution** into each well

8. **Incubate for 10 min** at RT in the dark. At a room temperature higher than 25 °C the substrate incubation time should be shortened. The minimum substrate incubation time must be 5 minutes.

9. **Pipette 100 µl stopping reagent** into each well

10. **Measure at 450 nm** within the next 30 min after stopping

6. Interpretation of Results

To prove the test function the absorbance of the positive control serum has to be distinctly higher than the absorbance of the cut off control sample. The negative control results have to be lower than the cut off control results.

A patient serum with a measured absorbance which is distinctly higher than the absorbance of the cut off control sample possesses an enhanced level of anti-U1-snRNP antibodies.

A small enhancement of up to 20% of the absorbance indicates a corresponding antibody concentration which is still within the limit or it indicates a slightly positive reaction of the patient serum.

If a serum reacts positively in the cut off-test the concentration of the corresponding antibody should be detected using an IMTEC-test which works quantitatively (Enzyme Immunoassay for the Determination of Anti-U1-snRNP Antibodies - Cat.No.: TC 60022).

Precautions

For in vitro diagnostic use only.

The human Control Sera and the cut off-control 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.



IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10

D-13125 Berlin

GERMANY

Tel.: +49(0)30 94 89 36 00

Fax: +49(0)30 94 89 36 15