

Test Instructions

Enzyme Immunoassay for the Detection of Anti-Scl-70 Antibodies (cut off - test)

Catalogue-No.: TC 70028

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 (ANA) are autoantibodies with different specificities which are directed against antigens of the cell nucleus. In general ANA are divided into antibodies against extractable nuclear antigens, nonextractable nuclear antigens and cytoplasmically located antigens. Anti-Scl 70 antibodies belong to a group of antibodies which are directed against extractable nuclear antigens.

First Scl 70 was described as a protein with a molecular weight of 70 kDa which was isolated from calf thymus cells. Later it has been shown, that the 70 kDa-protein is a degradation product of the native protein DNA-Topoisomerase I with a molecular weight of 100 kDa.

Autoantibodies directed against Scl 70 are almost exclusively found in patients with systemic scleroderma with a frequency of 70 %. Therefore such antibodies are considered to be marker antibodies of systemic scleroderma. On the other hand autoantibodies against Scl 70 are not detectable in a CREST syndrome which has a better prognosis than systemic scleroderma.

However, it is not always possible to differentiate between localized and systemic forms of scleroderma and that is why there is only a restricted possibility to relate sections of autoantibodies to clinical pictures.

2. Principle of the Test

The test is based on the immobilisation of Scl 70-antigen purified by affinity chromatography to a solid phase (polystyrene) and subsequent binding of the anti-Scl 70-anti-bodies. 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 ready to use buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2 Preparation of Sample Buffer

Dilute 1 unit sample buffer concentrate with 4 units distilled water. Should any of the salts be crystallized they should be resolved before use. The ready to use 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 1 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) cut off-control and control sera into each well, for blanks use sample diluent buffer instead of serum dilution, seal wells with adhesive foil
2. **Incubate for 1 hour** at room temperature (RT)
3. **Rinse off the wells 3x** 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 3x** with min. 200 µl washing buffer per well
7. **Pipette 100 µl substrate solution** into each well

8. **Incubate for 10 minutes** 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-Scl 70 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-Scl-70 Antibodies - Cat.No.: TC 60028).

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