

Test Instructions

Enzyme Immunoassay for the Detection of Anti-ssDNA Antibodies

Catalogue-No.: TC 59005

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.

Store reagents at 2-8°C.

1. Clinical Use

Anti-ssDNA-antibodies are directed against the purine and pyrimidine bases of the DNA. They do not react with helical dsDNA because all purine and pyrimidine bases are directed toward the center of the helix and are unavailable for reaction with the antibody.

Autoantibodies directed against ssDNA are detected in about 87% of patients with systemic lupus erythematosus (SLE) in its active stage and in about 43% of patients who are in the inactive phase of the disease. Therefore this antibodies are important activity markers in SLE.

The anti-ssDNA-antibodies can also be found in patients with other collagenoses, like drug-induced LE (52%), juvenile rheumatoid arthritis (35-50%), chronic polyarthritis, autoimmune chronic-aggressive hepatitis (58%) and leucemic diseases (60-89%). It is evident, that antibodies against ssDNA can be found in 25% of patients with collagenoses who give negative results in ANA detection.

Anti-ssDNA-antibodies are therefore an important criterion in the differential diagnosis of collagenoses.

2. Principle of the Test

The test is based on the covalent binding of a high purified ssDNA to microtiter strips which are chemical activated (patent pending) and subsequent binding of ssDNA antibodies from patient serum. The covalent binding of ssDNA to the solid phase prevents the refolding of DNA and the development of helical structures so that anti-dsDNA-antibodies are not detectable.

The bound antibodies are detected with an peroxidase-labeled secondary antibody 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

- ssDNA coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- standards, ready to use	1 vial each
1: 12.5 U/ml	750 µl per vial
2: 25 U/ml	
3: 50 U/ml	
4: 100 U/ml	
5: 200 U/ml	
(all standards contain 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
- HRP-Conjugate, anti-human IgG, concentrate (100x)	1 vial 200 µl
- HRP substrate (TMB), ready to use	1 bottle 12 ml
- stopping solution, contains 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

Dilute 1 unit sample buffer concentrate with 4 units distilled water. If any salt has been crystallized inside the bottle, it must be resolved before use. The diluted 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 samples freshly collected or freeze samples at – 20 °C. Allow the samples to reach room temperature (30 min). Dilute samples 1 : 100 with sample 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 sealed in the lockable original bag at 2 - 8 °C.

4.9. Stopping Solution

H₂SO₄ (caution !)

5. Test Procedure

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

- **Pipette 100 µl substrate solution** into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation could be shortened, but should never fall short of 5 minutes.
- **Pipette 100 µl stopping solution** per 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 U/ml, 25 U/ml, 50 U/ml, 100 U/ml, 200 U/ml) in semi log. Determine the units of the examined sera from the standard curve directly.

Results above 35 U/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 testkit.

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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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

imtec@mdc-berlin.de www.imtec-berlin.de