

Test Instructions

Enzyme Immunoassay for the Detection of Anti-dsDNA Antibodies

Catalogue-No.: TC 59001

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

The measurement of autoantibodies against double-stranded DNA (dsDNA) is one important criterion for the diagnosis of systemic lupus erythematosus (SLE). In the remission resp. in an immunosuppressive therapy the titer may dramatically decrease, whereas exacerbations are accompanied by an increase in anti-dsDNA antibodies. So the measured concentration of dsDNA antibodies gives helpful hints for prognosis and therapy of SLE-patients.

2. Principle of the Test

The test is based on the covalent binding of high purified dsDNA to chemical activated microtiter strips (patent pending) and subsequent binding of dsDNA antibodies from patient serum.

The bound antibodies are detected with a peroxidase-labeled secondary antibody that is directed against human IgG. After addition of substrate solution, a color stain develops and its intensity is proportional to the concentration and/or the avidity of the detected antibodies.

3. Material Provided

- dsDNA coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- standards, ready to use	1 vial each
1: 12.5 WHO-IU/ml	750 µl per vial
2: 25 WHO-IU/ml	
3: 50 WHO-IU/ml	
4: 100 WHO-IU/ml	
5: 200 WHO-IU/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

Please use sera but **no plasma samples** in the test. Use sera freshly collected or freeze samples at -20 °C. Allow the sera to reach room temperature (30 min). Dilute sera 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 WHO-IU/ml, 25 WHO-IU/ml, 50 WHO-IU/ml, 100 WHO-IU/ml, 200 WHO-IU/ml) in semi log. Determine the units of the examined sera from the standard curve directly. Results below 25 WHO-IU/ml (cut off value) are considered negative. Results from 25 - 40 WHO-IU/ml indicates a slightly positive reaction of the patient serum. Results above 40 WHO-IU/ml 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