

Test Instructions

Enzyme Immunoassay for the Detection of Anti-Nucleosome Antibodies

Catalogue-No.: TC 59002

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

Systemic lupus erythematoses (SLE) is characterized by the occurrence of a number of autoantibodies against various components of the nucleus, cytoplasmic antigens and cell membranes. Anti-dsDNA antibodies, which in 1982 were incorporated in the revised ACR criteria for this disease, are of great diagnostic and pathogenetic importance.

The detection of anti-nucleosome antibodies has gained increasing significance in SLE diagnostics in recent years.

Nucleosome antibodies are directed against the subnucleosomal complexes, a structural component of chromatin. The nucleosome itself consists of core particles containing the dimeric histones H2A-H2B and H3-H4, around which helical DNA containing 146 base pairs is coiled. Consequently, nucleosome antibodies can also encompass dsDNA and histone antibodies (except for histone-1 antibodies), similar to nucleosome-specific antibodies which, however, can only bind to a quaternary epitope of the complex.

Nucleosome antibodies are regarded as a diagnostic marker for SLE, detecting the disease with a sensitivity of 70 to 90%. The antibodies can be detected in almost 100 % of all patients with active SLE and in 62 % of those with inactive SLE (the corresponding frequency of detection of dsDNA antibodies in SLE is only 3.3 %). Hence – nucleosome antibodies are more sensitive for SLE than dsDNA antibodies - nucleosome antibodies are even detectable in dsDNA-antibody-negative patients. Nevertheless, a nucleosome antibody test may still be negative while a dsDNA antibody test is positive.

Nucleosome antibodies are also measurable in drug-induced lupus erythematoses. They are regarded as an early marker of the exacerbation of SLE, since they occur sooner than dsDNA antibodies.

2. Principle of the Test

The test is based on the binding of high purified nucleosome on microtiter strips and subsequent binding of anti-nucleosome antibodies from patient serum. The bound antibodies are detected with an peroxidase-labeled secondary antibody which is directed against human IgG.

After the addition of substrate solution, a color stain develops. Its intensity is proportional to the concentration and/or the avidity of the detected autoantibodies.

3. Material Provided

- 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 thiomersal)	1 bottle 50 ml
- sample buffer concentrate (5x), (contains sodium azide)	1 bottle 22 ml
- Conjugate buffer, ready to use (contains thiomersal)	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 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 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 sera or freeze samples at -20 °C. Allow the sera to reach room temperature (30 min). Dilute sera 1 : 100 with sample buffer (10 l serum to 1 ml buffer).

4.6. Preparation of Conjugate

The amount of conjugate dilution required daily, is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate dilution. Dilute conjugate 1:100 with conjugate 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. The opened substrate bottle should be closed carefully. Store substrate solution at 2 – 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 serum dilution** or (undiluted) standards and control sera into each well, for blanks 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** using at least 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** using at least 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.

Values below 25 U/ml (cut off) are negative. Results above 25 U/ml are considered positive.

To prove the functionality of the test, the value determined 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