

Test Instructions

Enzyme Immunoassay for the Detection of Antinuclear Antibodies (ANA) (cut off)

Catalogue-No.: TC 70001

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

Antinuclear antibodies (ANA) are autoantibodies with different specificities against antigens of the cell nucleus. The detection is important for diagnosis of collagenosis especially of systemic lupus erythematosus (SLE) and the mixed connective tissue disease (MCTD) which is strongly associated with it, and also of other rheumatic diseases. In SLE patients and MCTD patients ANA are detectable at almost 100%. From this follows, that the absence of these antibodies practically excludes a diagnosis of these. A positive detection of ANA is on the other hand not a proof for these diseases because ANA are detectable in other diseases, too.

2. Principle of the Test

The test is based on the covalent binding of HeLa cell nuclei, carefully isolated by density gradient centrifugation, to the solid phase of microtiter strips and subsequent binding of ANA from patient serum. The bound antibodies are detected with an peroxidase-labeled secondary antibody that is directed against human IgG and IgM.

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

3. Materials Provided

- coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- cut off control serum, lyophilized, ready to use, (contains sodium azide)	1 vial 2 ml
- positive control serum, ready to use (contains sodium azide)	1 vial 2 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 Ig(G/A/M) 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

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 the control sera

The cut off control sera and the control sera are ready to use.

4.4. Preparation of Sera

Allow the samples to reach room temperature (30 min). Dilute samples 1:100 with sample dilution buffer (10 µl sample to 1 ml buffer).

4.5. 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 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.

4.6. 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.

4.7. Microtiter Strips

The strips are ready to use. Unused strips should be stored in the lockable original bag at 2 - 8 °C.

4.8. 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 dilution 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

To prove the functionality of the test, the absorbance of the positive control serum has to be distinctly higher than the absorbance of the cut off control sample. The negative control result has to be lower than the cut off control result.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut off control sample possesses an enhanced level of antinuclear antibodies.

A small enhancement of up to 20% in the absorbance indicates a corresponding antibody concentration that 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 obtained result should be quantified using the quantitative ANA-Elisa (Catalogue-No. 60001) and the responsible antibody activity should be identified using the ANA/ENA-Profile-Test (Catalogue-No.: TC 60034 detecting ANA, dsDNA, SS-A/Ro, SS-B/La, Sm, U1-snRNP, Scl 70 and Histon).

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.



modified 03/2002

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