

Test Instructions

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

Catalogue-No.: TC 70031

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

Histons are basic nuclear proteins, which can complex with DNA. Anti-histone antibodies produce frequently a homogeneous pattern in indirect immunofluorescence assay. The specific estimation of these antibodies is realized with enzyme immunoassay techniques.

Anti-histone antibodies are especially detected in 95% of patients with drug-induced lupus erythematosus. These antibodies are particularly directed against H2A, H2B and H2A-H2B complex. Drug-induced anti-histone antibodies are also found in 19% to 30% of patients without symptoms of LE. These patients often develop a drug-induced SLE.

Anti-histone antibodies are detectable in 20% to 50% of SLE patients. These antibodies are also found in approximately 15% to 20% of patients with rheumatoid arthritis, in approximately 20% of patients with juvenile rheumatoid arthritis and with lower frequency in patients with other collagenous diseases. Anti-histone antibodies are also detected in approximately 60% of patients with primary biliary cirrosis. Furthermore these antibodies are also described for malignant diseases in approximately 10% of cases.

Anti-histone antibodies are important for the diagnosis of drug-induced and idiopathic lupus erythematosus despite the reduced specificity.

2. Principle of the Test

The test is based on the covalent binding of histone purified by chromatography to the solid phase of chemical activated microtiter strips (patent pending) and subsequent binding of the anti-histone antibodies. For the detection of antibodies bound in this way a peroxidase-labelled antibody is used, 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)	12 strips
- cut off control, ready to use, (contain sodium azide)	1 vial 2 ml
- negative control serum, ready to use, (contain sodium azide)	1 vial 1 ml
- positive control serum, ready to use, (contain sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x), (contain thimerosal)	1 bottle 50 ml
- sample buffer concentrate (5x), (contain sodium azide)	1 bottle
- 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

If any of the salts have been crystallized they should 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 one unit sample buffer concentrate with 4 units distilled water. If any of the salts have been crystallized they should be resolved before use. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.3 Preparation of the cut of 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 one 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) standards and control sera into each well, for blanks use ready to use dilution buffer instead of serum dilution, seal wells with adhesive foil
2. **Incubate for 1 hour** at room temperature (RT)
3. **Rinse off the wells 3 x** 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 3 x** with min. 200 µl washing buffer per well
7. **Pipette 100 µl substrate solution** into each well

8. **Incubate for 10 min** 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-histone 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-histone Antibodies, Cat.No.: TC 60031).

Precautions

For in vitro diagnostic use only.

The human Control Sera 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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