

Test Instructions

Enzyme Immunoassay for the Detection of Anti-CENP-B Antibodies

Catalogue-No.: TC 60005

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.

1. Clinical Use

The discovery of anti-centromere antibodies (ACA) was possible thanks to the use of the rapidly splitting HEP-2 cell line in indirect immunofluorescence. Autoantibodies to centromere antigens are directed against the inner and outer layers of kinetochores, but not against DNA.

The proteins CENP-A (19 kD), CENP-B (80 kD), and CENP-C (140 kD) are the three main target antigens. The CENP-B antigen plays a dominant role, since it is recognized by virtually all ACA's.

Because centromere antigens are sparingly soluble proteins that are bound to nuclear matrix proteins, it is very difficult to isolate and purify them. It is therefore becoming more and more popular to use a pro- or eukaryotically expressed C-terminal protein fragment composed of 147 amino acids, representing the immunodominant epitope of CENP-B.

Anti-centromere antibodies are primarily associated with limited forms of progressive systemic sclerosis (PSS), particularly CREST syndrome (calcinosis cutis, Raynaud's phenomenon, esophageal motility dysfunction, sclerodactylia, telangiectasia); an incidence of 50 – 70 % is reported. ACA's appear to signalize a favorable prognosis of PSS. Pulmonary, cardiac and renal manifestations are thus seldom observed in CREST syndrome.

Anti-centromere autoantibodies can be detected in 25 % of all patients with Raynaud's phenomenon. This is prognostically significant in these patients, because ACA's are associated with an increased risk of collagenosis.

Anti-centromere autoantibodies are detected in 10 – 20 % of all patients with primary biliary cirrhosis, but are much less frequent in patients with isolated pulmonary hypertension, SLE or primary Sjogren's syndrome.

2. Principle of the Test

The test is based on the immobilisation of recombinant CENP-B antigen to a solid phase (polystyrene) and subsequent binding of the anti-centromere antibodies. For the detection of autoantibodies bound to the solid phase, a peroxidase-labeled antibody is used that is directed towards human IgG. After addition of a peroxidase-

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

- microtiter strips (1 x 8), breakable, coated with CENP-B antigen	12 strips
- standards; ready to use,	1 vial each
1: 12.5 U/ml	à 750 µl
2: 25 U/ml	
3: 50 U/ml	
4: 100 U/ml	
5: 200 U/ml	
(containing 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,	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 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 salts has been crystallized it should 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

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 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 conjugate diluent buffer (for one plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining conjugate dilution should be disposed off.

4.7 Preparation of the Substrate

The TMB-substrate solution is ready to use. The opened substrate bottle should be closed readily after usage. 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 the wells 3 x** with a minimum of 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 the wells 3 x** with a minimum of 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 room temperatures above 25 °C the substrate incubation time could be shortened, but should never fall short of 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

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 semilog method. Determine the units of the examined sera from the standard curve directly.

Results above 25 U/ml (cut off value) are considered as 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 negative control has to be lower than the cut off value of the test.

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations that were **tested** for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies **with NEGATIVE results**.

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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