

# Test Instructions

## Enzyme Immunoassay for the Detection of Anti-SS-B/La Antibodies

Catalogue-No.: TC 60024

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

Antinuclear antibodies (ANA) are autoantibodies with different specificities which are directed against antigens of the cell nucleus.

In general ANA can be divided into antibodies directed against extractable nuclear antigens, nonextractable nuclear antigens and cytoplasmically located antigens. Antibodies against ribonucleoprotein containing nuclear substances like the SS-B/La-antigen belong to a group of antibodies which are directed against extractable nuclear antigens, but the SS-B/La-antigen are also found in cytoplasm.

Autoantibodies directed against the SS-B/La-antigen are especially detected in patients with a Sjogren's syndrome with a frequency of 85 %. Such autoantibodies are also detectable in patients with SLE, but only in 10 % to 20 % of cases.

Anti-SS-B/La-antibodies often occur together with antibodies which are directed against the SS-A/Ro-antigen.

Only in rare cases of other rheumatic diseases antibodies against the SS-B/La-antigen are detectable, in most cases they are not traceable.

Therefore antibodies against the SS-B/La-antigen possess a limited specificity for a Sjogren's-Sicca symptomatic complex.

### 2. Principle of the Test

The test is based on the immobilisation of recombinant produced SS-B/La-antigen to a solid phase (polystyrene) and subsequent binding of the anti-SS-B/La-antibodies. For the detection of antibodies bound in this way a peroxidase-labelled antibody is used which is 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. For antibody quantification the standard sample has been calibrated with the CDC-serum num. 2.

### 3. Material Provided

- coated microtiter strips (1 x 8), breakable	12 strips
- standards; ready to use,	1 vial each à 750 µl
1: 12.5 U/ml	
2: 25 U/ml	
3: 50 U/ml	
4: 100 U/ml	
5: 200 U/ml	
(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,	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 <sub>2</sub> SO <sub>4</sub> , 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 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 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 1 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

H<sub>2</sub>SO<sub>4</sub> (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

Calibrate measured absorbance against concentrations / units of standards ( 12.5 U/ml, 25 U/ml, 50 U/ml, 100 U/ml, 200 U/ml), (linear or semilog). Read the units of the examined sera from the standard curve directly.

Results above 25 U/ml (cut off value) are considered positive.

To prove the test function the value of the positive control serum has to be within the range (see label on the vial). The negative control result 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 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.



### 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