

# Test Instructions

## Enzyme Immunoassay for the Detection of PM-Scl-Antibodies (cut off -Test)

Catalogue-No.: TC 70035

Please read the instructions carefully before testing.

Procedural precautions:

Store reagents at 2 – 8 °C

Do not use the reagents beyond the date of expiry.

Never mix reagents from different test kit lots.

Indications:

1. Suspicion of polymyositis/dermatomyositis,
2. Suspicion of polymyositis scleroderma overlap syndrome,
3. Suspicion of scleroderma,
4. Suspicion of scleromyositis in childhood,
5. Diagnosis/differential diagnosis of myositis of unclear origin.

### 1. Clinical Use

Anti PM-Scl antibodies (also known formerly as PM-1 antibodies) belong to the group of nucleolar antibodies and were first discovered in patients with polymyositis and polymyositis-scleroderma overlap syndrome in 1977.

PM-Scl antigen is the exosome, a complex of 11 to 16 proteins located in the nucleoplasm and granular part of the nucleolus. These proteins function as exoribonucleases during RNA processing. Their primary target antigens are a 100 kD protein (PM-Scl-100) and a 75 kD protein (PM-Scl-75). The rate of recognition by PM-Scl antibodies is 100% for the PM-Scl-100 protein and 50 to 60% for the PM-Scl-75 protein.

PM-Scl antibodies are found almost exclusively in patients with idiopathic myositis and/or myositis overlap syndrome or scleroderma. When found, they occur only solely but not in combination with other marker antibodies for myositis or scleroderma. PM-Scl antibodies can be detected in approximately 8 to 16% of patients with polymyositis/dermatomyositis, 10% with scleroderma, and 24% with polymyositis scleroderma overlap syndrome.

Of the overall group of patients with this autoantibody, approximately 50 to 70% have polymyositis scleroderma overlap syndrome, 20% have idiopathic myositis, and 10% have scleroderma. In addition to scleroderma and myositis, characteristic for the overlap syndrome are frequently arthritis/inflammatory arthropathy, eczematoid skin changes with rhagades and keratosis as well as Raynaud symptoms. Esophageal motility disturbances are found in 45% of the patients, and (mild) interstitial lung changes are observed in 44%. Cardiac and renal changes are very rare. Consequently, the prognosis of the disease is relatively good.

### 2. Principle of the Test

The test is based on the immobilisation of recombinant PM-Scl 100 to a solid phase (polystyrene) and subsequent binding of the anti-PM-Scl 100-antibodies. For the detection of antibodies bound in this way a peroxidase-labeled antibody is used that is directed against human IgG. After addition of substrate solution, a color stain develops, the intensity of it is proportional to the concentration and/or the avidity of the antibodies.

### 3. Material Provided

- PM-Scl 100 coated microtiter strips (1 x 8), breakable	12 strips
- cut off control, ready to use, (contains sodium azide)	1 vial 2 ml
- 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 dilution buffer concentrate (5x),	1 bottle 22 ml
- conjugate dilution buffer concentrate (5x),	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 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 Dilution Buffer

Dilute 1 unit sample dilution buffer concentrate with 4 units distilled water. If any salt has been crystallized inside the bottle it must be resolved before use. The diluted sample buffer is stable for 6 weeks stored at 2 – 8 °C.

##### 4.3. Preparation of the Cut off Control

The cut off control is ready to use.

##### 4.4. Preparation of Control Sera

The control sera are ready to use.

##### 4.5. Preparation of Sera

Use serum samples freshly collected or freeze the samples at – 20 °C. Allow sera to reach room temperature (30 min). Dilute sera **1 : 100** with sample dilution 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 for preparation of the conjugate. Dilute peroxidase conjugate 1 : 100 with ready to use conjugate 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 off.

##### 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

- **Pipette 100 µl serum dilution** or (undiluted) standards or control sera into each well, for blanks

use ready to use dilution buffer instead of serum dilution, seal wells with adhesive foil

- **Incubate for 1 hour** at room temperature (RT)
- **Rinse wells 3 x** with min. 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 wells 3 x** with min. 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 time could be shortened, but should never fall short of 5 minutes.
- Pipette 100 µl stopping reagent into each well
- **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 result of negative control has to be lower than the cut off value of the test

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 specific antibodies (positive).

A small enhancement of up to 20 % of the absorbance indicates a slightly positive reaction of the patient serum only.

#### 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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#### 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