

# Test Instructions IMTEC-PM-Scl (cut-off)

## Enzyme Immunoassay for the Detection of Anti-PM-Scl Antibodies

REF : TC 70035

Please read the instructions carefully before testing.

**Procedural precautions:**

- ▶ Do not use the reagents beyond the date of expiry.
- ▶ Never mix reagents from different lots.
- ▶ Store reagents at 2-8°C.

### 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.

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.

### 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 to the microtiter plate an antibody directed to human IgG, conjugated with peroxidase, is used. After addition of peroxidase substrate solution, a color stain develops, its intensity is proportional to the concentration and/or the avidity of the anti-PM-Scl 100 antibodies.

### 3. Materials Provided

-	<b>MTP</b> : PM-Scl 100 coated microtiter strips (1x8), breakable	12 strips
-	<b>CONTROL</b> <b>co</b> : cut-off control, ready to use, contains sodium azide	1 vial 2 ml
-	<b>CONTROL</b> <b>-</b> : negative control serum ready to use, contains sodium azide	1 vial 1 ml
-	<b>CONTROL</b> <b>+</b> : positive control serum, ready to use, contains sodium azide	1 vial 1 ml
-	<b>BUF</b> <b>WASH</b> <b>10x</b> : washing buffer concentrate (10x)	1 bottle 50 ml
-	<b>DIL</b> <b>SPE</b> <b>5x</b> : sample buffer concentrate (5x)	1 bottle 22 ml
-	<b>CONJ</b> <b>a(hum IgG):HRP</b> : HRP-Conjugate, anti-human IgG, ready to use	1 bottle 12 ml
-	<b>SUBS</b> <b>TMB</b> : TMB solution, HRP substrate, ready to use	1 bottle 12 ml
-	<b>SOLN</b> <b>STOP</b> : stopping solution, ready to use, contains sulfuric acid, caution corrosive!	1 bottle 12 ml

## 4. Preparation of Reagents

### Attention!

Allow the testkit and all its components to reach room temperature completely before executing it !

Please do not use any polystyrene vessels for handling of HRP conjugates.

If the test is performed automatically, we recommend the use of fresh conjugate each run and to discharge traces of old conjugate entirely. Remove washing buffer after washing steps completely.

### 4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 part washing buffer concentrate [BUF] [WASH] [10x] with 9 parts 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 part sample buffer concentrate [DIL] [SPE] [5x] with 4 parts distilled water. The diluted buffer is stable for 6 weeks stored at 2-8°C.

### 4.3. Control Sera, HRP Conjugate, Stopping Solution and TMB Solution

Control sera, HRP conjugate, stopping solution and TMB solution are ready to use. Used bottles should be closed carefully and stored at 2-8°C. **Store TMB solution also protected from light.**

### 4.4. Preparation of Sera

Use serum samples freshly collected or freeze samples at - 20 °C. Allow sera to reach room temperature (30 min). Dilute sera 1 : 100 with sample buffer (10 µl sample to 1 ml buffer).

### 4.5. Microtiter Strips

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

## 5. Test Procedure

- **Pipette 100 µL serum dilution** or control sera [CONTROL] [+], [CONTROL] [co] and [CONTROL] [-] into each well, for blank use sample 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.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µL of HRP-conjugate** [CONJ] [a(hum IgG):HRP] 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.
- **Discard buffer and knock out residues** on an absorbent paper or cloth.

- **Pipette 100 µL TMB solution** [SUBS] [TMB] 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** [SOLN] [STOP] 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 [CONTROL] [+] has to be distinctly higher than the absorbance of the cut-off control [CONTROL] [co]. The absorbance of the negative control [CONTROL] [-] has to be lower than the cut-off control [CONTROL] [co] of the test.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut-off control [CONTROL] [co] possesses an enhanced level of specific antibodies (positive).

### Precautions

For *in vitro* diagnostics only !

[IVD]

The human Control Sera and Standards in this kit have been prepared from blood donations.

However, as nobody 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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