Polymyositis/Scleroderma[8] IgG

Order Code: PMS8D-24

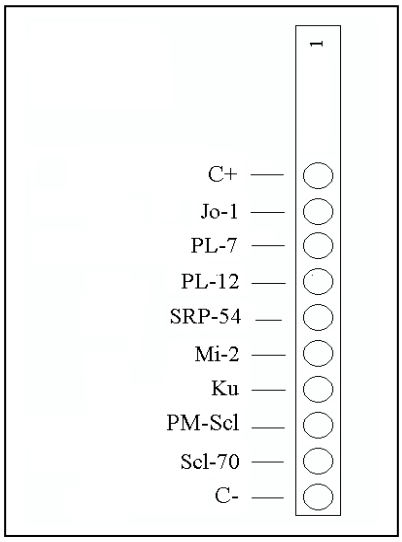
1. INTENDED USE

BlueDOT Polymyositis/Scleroderma[8] IgG is an Immunodot kit intended for the detection in human sera of IgG autoantibodies against Jo-1, PL-7, PL-12, SRP-54, Mi-2, Ku, Pm-Scl and Scl-70 antigens.

More information on the source/type of antigens is available via your distributor or via our website www.d-tek.be (MSDS).

2. PRINCIPLE OF THE TEST

The test is based on the principle of an Enzyme Immunoassay. The test strip is composed of a membrane fixed on a plastic support. During test procedure, the strips are incubated with diluted patients' sera. Human antibodies, if present, bind to the corresponding specific antigen(s) on the membrane. Unbound or excess antibodies are removed by washing and AP-conjugated goat antibodies against human IgG are added to the strips. This enzyme conjugate binds to the antigen-antibody complexes. After a second washing step to remove excess conjugate, substrate solution is added. Enzyme activity, if present, leads to the development of purple dots on the membrane pads. The intensity of the coloration is directly proportional to the amount of antibody present in the sample.



3. KIT CONTENTS

Abbreviations:
TBS = Tris Buffer Saline; BSA = Bovine Serum Albumin; MIT = MethylIsoThiazolone ;
AP = Alkaline Phosphatase ; NBT = NitroBlue Tetrazolium ; BCIP = Bromo-Chloro-Indolyl-Phosphate.

TO BE DILUTED :	**(10 x) Wash buffer**	**1 x 40 ml** (colourless) *Contains : TBS, Tween ; Preservative : MIT*
READY TO USE :	**Dot strips**	**24 units** *10 Dots each:* *1 negative control* **(C-)** *8 antigens* *1 positive control* **(C+)**
	Diluent buffer	**1 x 40 ml** (yellow) *Contains : TBS, BSA, Tween ; Preservative : MIT*
	Conjugate	**1 x 40 ml** (red) *Contains : AP-conjugated goat anti-human IgG ; Preservative: MIT*
	Substrate	**1 x 40 ml** (brown bottle, pale yellow solution) *Contains: NBT/BCIP; Preservative: 0.05 % NaN_3 (sodium azide)*
	Incubation trays	**3 units** *with 8 wells for incubation*

4. MATERIAL REQUIRED BUT NOT PROVIDED

Rocking or shaking platform / Micropipettes / Timer / Graduated cylinder / Distilled or deionised water / Tweezers / Absorbent and/or filter paper

5. STORAGE

The reconstituted Wash Solution is stable for at least one month at 2-8°C. Reagents and strips can be stored at 2-8°C until the expiry date indicated on each vial or tube.

Place unused strips back into the provided tube, seal it and store at 2-8°C. Chromogen/Substrate (NBT/BCIP) shall be stored at 2-8°C.

6. PRECAUTIONS

All reagents are for in vitro diagnostic and professional use only. The kit contains potentially hazardous components thus avoid contact with skin, eyes or mucosae. Patient samples shall be handled as potentially infectious. Do not substitute reagents or mix strips with different batch numbers this may lead to variations in the results. Avoid touching strips with fingers. Use tweezers or wear laboratory gloves. Allow reagents and strips to equilibrate at room temperature before use. Strictly observe incubation times. Handle Chromogen Substrate (NBT/BCIP) with care in order to avoid any contamination with Alkaline Phosphatase.

7. SAMPLE COLLECTION, HANDLING AND STORAGE

Samples should be preferentially fresh-collected ones. Sera with debris should be low speed centrifuged. Blood samples should be collected in dry tubes or in tubes containing EDTA or heparin. After separation serum samples shall be used immediately or aliquoted and stored at 2-8°C for some days or frozen at -20°C for longer periods. Avoid repeated freezing thawing cycles.

8. ASSAY PROCEDURE

BASIC HANDLING AND TIPS:
The dots are precoloured blue on the strips, ensuring that all antigens have been dotted correctly onto the membrane. This **blue coloration disappears** during the first step of the incubation. During incubation with the wash buffer, a faint pink background coloration appears on the membrane and disappears upon drying at the end of the procedure.
During the procedure, **agitation** of the incubation tray is necessary to ensure efficient circulation of fluids over the membrane. A **Rocking platform** is the shaker of choice. Be sure to adjust the movement of the shaker in such a way that no spilling of solutions or cross-contamination between the wells can occur.
After each filling of the wells with solution, agitate manually the incubation tray until the strips are completely immersed in order to remove air bubbles which may be trapped under the strip. Alternatively, floating strips may be forced into the solution by pushing down (with tweezers or pipette tip) on the upper part of the strip (plastic label zone).

Avoid touching the membrane zone of the strip with fingers, tweezers or pipette tips. Always use the plastic label zone for handling or manipulation. The whole procedure has to be run **at room temperature.**

8.1 Reagents preparation

1. Allow all components to equilibrate at room temperature before use.
2. **Dilute** the concentrated **Wash Buffer 10x** with **distilled water.**
   Prepare 15 ml diluted Wash buffer per strip tested
   Example: 1,5 ml concentrated wash buffer + 13,5 ml distilled water for one strip.

8.2 Pipetting flow chart

1. **Place** one **strip** per patient into the wells, blue dots **facing up**.
2. Add **2 ml Wash Buffer** per well. **Incubate** (shake) **for 10 min.**
   Upon correct incubation the blue coloration of the dots completely disappears.
   If not prolong the procedure until the colour of the dots fades completely.
3. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
4. Add **1,5 ml Sample Diluent** per well.
5. Add **10 µl patient sample** per well. **Incubate** (shake) **for 30 min.**
   Avoid touching the membrane with the pipette tip. Preferentially dispense the sample into the solution over the upper part of the strip (plastic label zone).
   Note: Steps 4 and 5 can be combined by pre-diluting the sample in a glass or plastic tube (1,5 ml diluent + 10 µl patient sample (Mix (Add to the well)

6. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
7. **Wash 3 x 3 minutes** with **1,5 ml Wash Buffer** per well (shake)**.**
   Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper
8. Add **1,5 ml Conjugate** per well. **Incubate** (shake) **for 30 min**.
9. **Discard** solution from the wells.
   Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper
10. **Wash 3 x 3 min.** with **1,5 ml Wash Buffer** (shake)
    Following each wash step remove liquid from the wells by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edges of the tray with absorbent paper.
11. Add **1,5 ml Substrate** per well. **Incubate** (shake) **for 10 min.**
12. **Discard** solution from the wells.
    Remove liquid by slowly inverting the plate. The strips will adhere to the bottom of the wells. Dry the edge of the tray with absorbent paper.
13. **Wash 1 x 3 min.** with **1,5 ml Wash solution** per well to stop the reaction.
14. **Collect** the strips from the wells and allow them to dry on absorbent paper.

9. RESULTS INTERPRETATION

9.1 Interpretation

1. Peel off the cover of the adhesive on the back side of each strip and attach strips dots face up onto the marked fields of the interpretation sheet provided with the kit. This will indicate the respective positions of the different controls and antigens on the membrane.
2. **Check** the first upper Dot (**Positive control**): it must be positive for all patients.

   Only a clearly coloured positive Reaction Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded.
3. **Compare** the specific **antigen** Dots to the **Negative Control** Dot which always is the last in order.

   The colour intensity of the Antigen Dots is directly proportional to the titer of the specific antibody in the patients sample.

   Under optimum conditions and if the sample is free of interfering substances the negative control dot may be even close to uncoloured. In contrast highly coloured negative control dots indicate a high rate of unspecific binding in the sample.

POSITIVE RESULT: A sample is positive for a specific antibody if the colour intensity of the corresponding **Antigen** Dot is **higher** than the intensity of the **Negative Control Dot**.

NEGATIVE RESULT: A sample is negative for a specific antibody if the colour intensity of corresponding **Antigen** Dot is **lower or equal** than the intensity of the **Negative Control Dot.**

10. PERFORMANCES

10.1 Reproductibility

Remark: BlueDOT is a qualitative test and the precision of the assay is evaluated in terms of variation of the visual color intensity of the dots. The color intensity is estimated (from 0 to +5) by visual comparison with a reference color scale (membrane strip with 6 pre-colored reference dots).
Three control sera (High, medium, low) were assayed for intraassay and interassay imprecision in a statistically relevant repetition.
Detailed and updated data are available upon request.

10.2 Sensitivity and Specificity

Sensitivity: Jo-1, PL-7, PL-12, SRP-54, Mi-2, Ku, Pm-Scl, Scl-70 >99%.
Specificity: Jo-1, PL-7, PL-12, SRP-54, Mi-2, Ku, Pm-Scl, Scl-70 >99%.

Clinically defined populations (confirmed positive with disease specific reference methodologies) have been used for checking the sensitivity. Specificity was checked with control groups that embrace a normal healthy population as well as clinically defined control groups. Detailed data are available upon request.

11. TEST LIMITATIONS

1. A diagnosis should not be made solely on the basis of the test results.
2. Test results should always be interpreted in conjunction with the complete clinical evaluation and the results of other diagnostic procedures, only.
3. D-tek s.a. and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
4. In any case, GLP should be applied with all general and individual regulations to the use of this kit.

12. TROUBLE SHOOTING

No colour development	- Concentrated wash buffer used instead of diluted wash buffer - Samples over diluted - Conjugate diluted (ready to use) - Inactivated conjugate
Too high background	- Bad quality of serum : particles, old serum, bacterial contamination - The pre-wash step was insufficient or inadvertently omitted - Poor washing - Over incubation time - Over incubation temperature - Under diluted samples - Contaminated NBT

If for any reason outside of the operator's responsibility the kit should not perform as expected, please contact your supplier.

13. BIBLIOGRAPHY

Up to date literature is available upon request. Please inquire at info@d-tek.be