ALPHADIA S.A. / N.V.
DIAGNOSTIC PRODUCTS

CE

ENA Screen DOT KIT

Sm-Sm/RNP-SSA-SSB-JO1-Scl70-PMScl-CENP B
24 Tests - Code: AD ENASD
For in vitro use only

1. INTENDED USE

ALPHADIA ENA Screen DOT kit is an Immunodot kit intended for the detection in human sera of IgG autoantibodies against Sm, Sm/RNP, SSA (Ro), SSB (La), Jo-1 (histidyl-t-RNA synthetase), Scl-70 (DNA topoisomerase I), PM-Scl 100 and CENP-B (centromere B protein) antigens.

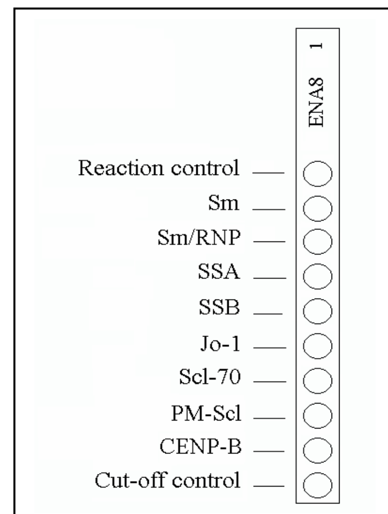
2. INTRODUCTION

Antinuclear autoantibodies (ANA) have been described very early and clinical associations between specific autoantibodies and autoimmune diseases have been increasingly investigated over years. Nuclear antigens are generally classified into subgroups based on their differential solubility in saline buffers:
ENA (Extractable Nuclear Antigens) are soluble in physiological buffers, a property that makes them identifiable by Immunodiffusion or Counterimmunoelectrophoresis (CIE) against a nuclear extract. The most common anti-ENA antibodies include anti-Sm, RNP, SSA (Ro), SSB (La), Jo-1, Scl-70 and PM-Scl. They are considered major autoimmune markers and have been widely used in a variety of techniques for the differential diagnosis of connective tissue diseases.
NON-EXTRACTABLE nuclear antigens are defined as being non-soluble in physiological conditions. As shown by Immunofluorescence patterns, most of them are closely linked to the chromatin structure and their isolation generally requires laborious purification techniques. Both Immunodiffusion and CIE are unsuitable methods for detecting the corresponding antibodies and only the molecular cloning allowed the commercialisation of specific immunoassays based on recombinant antigens (e.g the centromere antigen CENP-B).
The following table displays major disease associations of ENA and CENP-B autoantibodies

Antibody	Disease
Sm	Systemic Lupus Erythematosus (SLE)
RNP	Mixed Connective Tissue Disease (MCTD)
SSA (Ro)	Sjögren's Syndrome, SLE
SSB (La)	Sjögren's Syndrome
Jo-1	Polymyositis, Dermatomyositis
Scl-70	Systemic Sclerosis (SSc)
PM-Scl	Myositis/SSc overlap
CENP-B	CREST Syndrome

3. 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.



4. 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 50 ml** (colourless)
Contains : TBS, Tween ; Preservative : MIT

READY TO USE :

Dot strips : **24 units**
(10 Dots each: 1 reaction control, 8 antigens, 1 cut-off control)

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)

5. MATERIAL REQUIRED BUT NOT PROVIDED

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

6. 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.

7. PRECAUTIONS

All reagents are for in vitro diagnostic 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.

8. 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.

9. 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 procedure. During incubation with the sample 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. Any kind of shaking or rocking platform may be used if the amplitude of the movement does not cause spilling out of solutions and cross-contamination between the wells. Agitation speeds ranging from 10 to 60 rpm are suitable, with 20–30 rpm being preferred.

After each filling of the wells with solution, agitate manually the incubation tray to ensure that the strips are completely immersed and 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

9.1 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.

9.2 TECHNIQUE

1. Place one strip per patient into the wells, blue dots facing up.

2. Add 2 ml Wash Buffer to each 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 edges of the tray with absorbent paper.

4. Add 1,5 ml Sample Diluent to the wells.

5. Add 10 µl patient sample to the wells. Incubate (shake) for 30 min.
   Avoid touching the membrane with the pipette tip. Preferentially dispense the sample in the solution over the upper part of the strip (plastic label zone).

<u>Note:</u> *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. Wash 3 x 3 minutes with 1,5 ml Wash Buffer (shake).
   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.

7. Add 1,5 ml Conjugate to each well. Incubate (shake) for 30 min.

8. Wash 3 x 3 min. with 1,5 ml Wash Buffer (see 6.)

9. Add 1,5 ml Substrate to the wells. Incubate (shake) for 10 min.

10. Wash 1 x 3 min. with 1,5 ml Wash solution to stop the reaction.

11. Collect the strips from the wells and allow them to dry on absorbent paper.

10. RESULTS INTERPRETATION

10.1 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 (reaction 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 cut-off 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 cut-off dot may be even close to uncoloured. In contrast highly coloured cut-off 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 cut-off 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 cut-off Dot.

11. TEST LIMITATIONS

ALPHADIA ENA Screen DOT kit is intended for the detection of IgG antibodies to extractable nuclear antigens (including PmScl) and CENP-B protein. it may serve as an aid for the differential diagnosis of connective tissue diseases. However results shall be interpreted in the light of a complete clinical examination and case history always.

12. BIBLIOGRAPHY

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