ENA$^{+Nucleosome}$ IgG

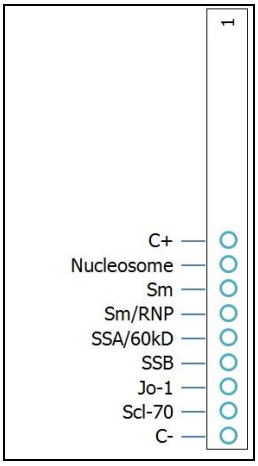
Order Code: NUENAD-24

1. INTENDED USE

BlueDot ENA$^{+Nucleosome}$ IgG is an Immunodot kit intended for the detection in human sera of IgG autoantibodies against Nucleosome, Sm, Sm/RNP, SSA/Ro 60kD, SSB(La), Jo-1 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:

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

TO BE DILUTED :	**(10 x) Wash buffer**	**1 x 40 ml** (colourless) *Contains : TBS, Tween; Preservative: MIT*
READY TO USE :	**Dot strips**	**24 units** *9 dots each:* *1 negative control* **(C-)** *7 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 for 30 minutes on absorbent paper. The interpretation has to be done in the 24 hours following the test processing.

9. RESULTS 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. The first upper dot (Positive Control Dot) must be positive for all patients. Only a clearly coloured Positive Control Dot ensures your results are valid and operation was correct and/or kit components were not degraded. If the first upper dot is not coloured, the test has failed and cannot be interpreted further.
3. Compare the specific antigen dots to the Negative Control Dot (which always is the last bottom dot). The colour intensity of the antigen dots is directly proportional to the titer of the specific antibody in the patient sample.
   The colour intensity of the Negative Control Dot may vary depending on the sample characteristics. If the sample is free of interfering substances the Negative Control Dot may be even close to uncoloured. In contrast, a highly coloured Negative Control Dot indicates 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 than or equal to the intensity of the Negative Control Dot.

NB: A weak coloration of an antigen dot, when close to the color intensity of the Negative Control dot may be difficult to differentiate by visual inspection only. In such cases, it is recommended to use Dr DOT software and scanning system (for more information on the Dr DOT software, please contact your distributor) and refer to the corresponding instructions for more accurate interpretation.

Dr DOT arbitrary unit (AU)	Interpretation
< 5	Negative
5 – 10	Equivocal (*)
>10	Positive

** Low titers of auto-antibodies may occur in healthy patients. For this reason low positive results (results comprised between 5 to 10 AU), although valid, are less reliable than results which are further than the cut-off (> 10 AU). Retesting of the patient, preferably by using a new sample, is therefore recommended. If the result is still equivocal on retesting, then other diagnostic tests and/or clinical information should be used to help determine the autoimmune status of the patient.*

10. PERFORMANCES

10.1 Reproductibility

Reference control samples were tested for each antibody in statistically relevant repetitions in a same run or over several runs for the calculation of intra- and inter-assay variation, respectively. In every case the intensity of the dots were within the specified range and standard deviations were less than 10 % *Detailed analytical data are available upon request.*

10.2 Sensitivity and Specificity

Characterized samples (confirmed positive or negative for specific antibodies by reference laboratories and/or methodologies) were assayed following the test instructions. Sensitivity and Specificity were calculated from the results generated by the Dr DOT software.

Nucleosome

	+	-
+	true positive /	false positive /
-	false negative /	true negative /

Sensitivity - %
Specificity - %
No reference method !

Sm

	+	-
+	true positive 36	false positive 2
-	false negative 0	true negative 100

Sensitivity 100%
Specificity 98%

Sm/RNP

	+	-
+	true positive 24	false positive 0
-	false negative 0	true negative 30

Sensitivity 100%
Specificity 100%

SSA/Ro60kD

	+	-
+	true positive 69	false positive 0
-	false negative 1	true negative 78

Sensitivity 99%
Specificity 100%

SSB

	+	-
+	true positive 54	false positive 1
-	false negative 0	true negative 93

Sensitivity 100%
Specificity 99%

Jo-1

	+	-
+	true positive 22	false positive 0
-	false negative 0	true negative 119

Sensitivity 100%
Specificity 100%

Scl-70

	+	-
+	true positive 13	false positive 0
-	false negative 0	true negative 91

Sensitivity 100%
Specificity 100%

➔ *For the antigen in grey: since no clearly characterized sample nor reference method is currently available, no values can reasonably be claimed.*

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 authorised distributors shall not be liable for any damages resulting from a change or modification in 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.
5. D-tek's liability shall in any event be limited to the replacement of the 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.

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