DRG® Blot Nucleo Screen (DOT-5123 & DOT-5124)

RUO in the USA

As of 26 Jan. 2011 rm (Vers. 1.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

1 NAME AND INTENDED USE

Nucleo Screen Blot is a membrane based enzyme immunoassay for determination of IgG class autoantibodies to nuclear antigens: dsDNA, nucleosomes, SS-A, SS-B, Sm, RNP/Sm, Scl-70, Jo-1 and centromere B.

SUMMARY AND EXPLANATION OF THE TEST

The superior sensitivity and specificity of the immunoblot system is achieved by using purified native or recombinant antigens and makes it an important diagnostic tool in the clinical laboratory for ANA detection (Carey, 1997).

Disease association of the most common Anti-Nuclear Antibodies (ANAs):

2 PRINCIPLE OF THE TEST

Highly purified nuclear antigens are bound to nitrocellulose membrane strips. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the membrane strips removes unspecific serum and plasma components. Alkaline phosphatise conjugated anti-human IgG immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the membrane strips removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyses to form an insoluble blue-violet product. Washing of the membrane strips removes unhydrolysed substrate.

The intensity of the colour is directly proportional to the concentration of IgG antibodies present in the original sample.

3 WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for RESEARCH USE ONLY.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the substrate solution BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/p-nitro blue tetrazolium chloride). If BCIP/NBT comes into contact with skin, wash thoroughly with water and soap.
5. Some kit components (i.e. controls, sample buffer and buffered wash solution) contain sodium azide as preservative. Sodium azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 7., 8., 9.)..
6. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
7. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

8. Do not pipette by mouth.
9. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

4 CONTENTS OF THE KIT

Package size	8 or 16 determinations
Qty. 8 or 16	**Nitrocellulose strips**, loaded with highly purified native or recombinant nuclear antigens. Ready to use.
1 vial, 20 ml	**Sample buffer**. Ready to use. This buffer is specifically adapted for the Nucleo Screen Blot and is not interchangeable with sample buffers of other immunoblots.
1 vial, 20 ml	**Wash buffer**, concentrate (50x).
1 vial, 20 ml	**Enzyme conjugate** solution (PBS, NaN_3 <0.1 % (w/w)), (pink) containing polyclonal goat anti-human-IgG; labelled with alkaline phosphatase. Ready to use.
1 or 2 vials, 10 ml	**Substrate solution** (BCIP/NBT). Ready to use.
Qty. 1 or 2	**Pre-developed nitrocellulose calibration strip** (labelled CAL) for semi-quantitative evaluation. Ready to use.
Qty. 1 or 2	**Incubation tray**.
Qty. 1 or 2	**Documentation sheet**.

5 STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep nitrocellulose strips dry; store together with desiccant and carefully sealed in the plastic tube.
3. **Important:** The calibration strip is very light-sensitive. Store the strips in a dark place!
4. The reagents are stable until expiration of the kit.
5. Do not expose test reagents to heat, sun or strong light during storage and usage.
6. Wash buffer are stable for at least 30 days when stored at 2-8 °C.

6 MATERIALS REQUIRED

Equipment

- Pipettes for 10 µl and 1000 µl
- Laboratory timing device

- Rocking platform
- Tweezers

Preparation of reagents

- Distilled or deionised water
- Graduated cylinder for 1000 ml

7 SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolysed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

8 PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. To avoid carryover contamination, change the tip between samples and different kit controls.
8. Nitrocellulose strips must be handled with gloves or tweezers.
9. All incubation steps must be accurately timed.
10. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
11. It is important to make sure, that air-bubbles do not interfere with the strip during incubation. This could cause irregularities in coloration of developing bands and can lead to wrong results.

9 PREPARATION OF REAGENTS

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

10 TEST PROCEDURE

1. Insert a Nucleo Screen Blot strip using tweezers
   then add 1.0 ml sample buffer to each chamber of the incubation tray.
   Allow to equilibrate for **5 minutes** with gentle rocking.
2. Add 10 µl of patient serum directly to the chamber (effective dilution 1:101).
3. Incubate for **60 minutes at room temperature** (20-28 °C).
4. Carefully remove the diluted serum completely from the strips.
5. Add 2.0 ml wash buffer, incubate for **5 minutes**, and then remove as in step 4.
   Repeat this procedure twice.
6. Add 1.0 ml enzyme conjugate to each chamber.
7. Incubate for **30 minutes** with gentle rocking **at room temperature**.
8. Remove the diluted conjugate completely from the strips.
9. Add 2.0 ml wash buffer, incubate for **5 minutes**, and then remove as in step 4.
   Repeat this procedure twice.
10. Add 1.0 ml substrate to each strip.
11. Incubate for **10 minutes** with gentle rocking **at room temperature**.
12. Remove the substrate and wash the strips with 1 ml distilled water three times 5 minutes each to stop the reaction.
13. Carefully blot the strips dry with a paper towel.
14. Allow strips to air dry before evaluating.

11 LIMITATIONS OF PROCEDURE

The Nucleo Screen immunoblot assay is for Research Use Only.

12 INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera.

Nor have any interfering effects been observed with the use of anticoagulants.

However for practical reasons it is recommended that grossly hemolysed or lipemic samples should be avoided.

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INCUBATION SCHEME

1. Add **blot strip** into the incubation tray
   → Add **1000 µl** sample buffer per strip into the incubation tray
   → Shake **5 minutes** while incubating
2. Add **10 µl** patient sample and resuspend
   → Shake **60 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash
3. Add **1000 µl** enzyme conjugate solution per strip
   → Shake **30 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **2000 µl** wash buffer, discard wash
4. Add **1000 µl** substrate per strip
   → Shake **10 minutes** while incubating
   → Discard content and wash 3 times for **5 minutes** with **1000 µl distilled water**, dry blot strips. Read after complete drying, only