

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

## **1 NAME AND INTENDED USE**

ANA Screen Blot is a membrane based enzyme immunoassay for determination of IgG class autoantibodies to extractable nuclear antigens (ENAs) SS-A 52, SS-A 60, SS-B, RNP/Sm, Sm, Centromere B, Jo-1, Scl-70 and ribosomal P proteins.

This kit is intended for Research Use Only. Not for use in diagnostic procedures..

## **2 PRINCIPLE OF THE TEST**

Highly purified extractable 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 phosphatase conjugated anti-human IgG immunologically detects the bound 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. This kit is intended for Research Use Only. Not for use in diagnostic procedures.
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 ( $\text{NaN}_3$ ) 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.

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#### 4 CONTENTS OF THE KIT

Package size	8 or 16 determinations
Qty. 8 or 16	<b>Nitrocellulose strips</b> , loaded with highly purified native or recombinant nuclear antigens. Ready to use.
1 vial, 20 ml	<b>Sample buffer.</b> Ready to use. This buffer is specifically adapted for the ANA Screen Blot and is not interchangeable with sample buffers of other immunoblots.
1 vial, 20 ml	<b>Wash buffer,</b> concentrate (50x).
1 vial, 20 ml	<b>Enzyme conjugate</b> solution (PBS, NaN <sub>3</sub> <0.1 % (w/w)), (pink) containing polyclonal goat anti-human-IgG; labelled with alkaline phosphatase. Ready to use.
1 or 2 vials, 10 ml	<b>Substrate solution</b> (BCIP/NBT). Ready to use.
Qty. 1 or 2	<b>Pre-developed nitrocellulose calibration strip</b> (labelled CAL) for semi-quantitative evaluation. Ready to use.
Qty. 1 or 2	<b>Incubation tray.</b>
Qty. 1 or 2	<b>Documentation sheet.</b>

#### 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. Diluted sample buffer and 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

Revised 17 Nov. 2010 rm (Vers. 1.1)

## 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 an ANA 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 serum directly to the chamber (effective dilution 1:101).
3. Incubate for **60 minutes at room temperature** (20-28 °C).

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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 REFERENCES / LITERATURE**

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**DRG**



**DRG® Blot ANA Screen (DOT-5113 and DOT-5114)**



**RUO** in the USA

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