MONKEY LIVER/ CEREBELLUM IFA SLIDES

For In Vitro Research Use

THE BINDING SITE LTD
Telephone: +44 (0)121-414 2000
Fax: +44 (0)121-472 6017
E-mail: info@bindingsite.co.uk
www/bindingsite.co.uk

1 INTENDED USE

Monkeys cerebellum/liver frozen sections are intended for use in indirect immunofluorescence assays, screening human serum for circulating anti-Hu antibodies (ANNA-1) and anti-Yo antibodies (PCA-1). The liver allows for the detection of interfering anti-nuclear antibody patterns.

The Binding Site anti-human IgG (H&L) FITC conjugate (monkey adsorbed) (FA003.M) is recommended for use with these slides because the absence of non-specific tissue staining enhances the sensitivity and facilitates interpretation.

2 PRINCIPLE

These slides are used in an indirect immunofluorescence technique where patient samples and appropriate controls are incubated with the frozen sections. The unreacted antibodies are washed off and then appropriate fluorescent labelled conjugates are applied. Unbound conjugate is washed off as before. Slides are viewed with a fluorescence microscope and positive samples produce apple-green fluorescence which corresponds to areas of the frozen section where autoantibody has bound.

3 REAGENTS

Monkey cerebellum/liver sections on 5-well slides individually wrapped in a foil pouch containing a desiccant.

4 CAUTION

All human serum tested using these slides should be previously tested and found to be negative for Hepatitis B surface antigen and antibodies to Hepatitis C virus and Human Immunodeficiency Virus (HIV 1 & 2). However, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material and only personnel adequately trained in such methods should be permitted to perform the procedures.

5 STORAGE AND STABILITY

Unopened slides should be stored at 2-8°C and can be used until the given expiry date. DO NOT FREEZE. Once slides are removed from a foil bag, they should be used immediately.

6 SPECIMEN COLLECTION

Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 48 hours prior to assay, or for prolonged storage, aliquoted and stored at -20°C or below. DO NOT freeze and thaw sera more than once. Avoid using lipaemic, haemolysed or microbially contaminated sera as decreased titres or unclear staining patterns may occur.

7 METHODOLOGY

7.1 Materials provided

10x 5-well slides
1 x instruction leaflet.

7.2 Additional materials required

1. PBS for sample diluent and washes.
2. Container for PBS buffer
3. Micropipettes and disposable tips to apply patient samples
5. Fluorescence microscope with 495nm exciter filter and 515nm barrier filter
6. Plastic squeeze bottle for initial wash in PBS.
7. Additional components may be obtained from The Binding Site: PBS (CON 3.3), negative control (CON93), anti-Hu (ANNA-1) positive control (FA199), anti-Yo (PCA-1) positive control (FA198), anti-homogenous positive control (FA105), anti-Human IgG (H+L) monkey adsorbed conjugate (FA003.M) and 1% Evans’s Blue (CON 93), Mounting medium (CON195).

7.3 Test Procedure

Quality Control
Positive and negative controls should be used every time samples are tested.

1. Mounting Medium
   Remove the mounting medium from the fridge to allow it to reach room temperature (18-28°C) before it is needed.
2. Dilute Patient Samples
   A. Screening: Dilute patient samples 1/50 by adding 10µL of serum to 490µL of PBS buffer.
   B. Titration: Make serial dilutions of positive screened samples with PBS buffer (eg. 1/100, 1/200, 1/400 and 1/800 etc).
   For example: Take 100µL of the 1/50 dilution, mix with 100µL PBS to give a 1/100 dilution. (Repeat for further dilutions).
3. Substrate Slides
   Allow substrate slide(s) to reach room temperature (18°C-28°C) prior to removal from pouch(es).
   Label slides appropriately, place in the humid chamber and add one drop of each positive and negative control to appropriate wells. Add 50 – 100µL of neat patient samples to the remaining wells.
4. Slide Incubation
   Incubate slides for 30 minutes in a humid chamber at room temperature (18°C-28°C).
5. PBS Wash
   Remove slides from humid chamber and rinse briefly with PBS squeeze bottle. Do not squirt directly on to the wells. Place slides in a rack and immerse in PBS and agitate or stir for 5-10 minutes.
6. Addition of fluorescent conjugate
   Shake off excess PBS and blot around wells. Return slides to humid chamber and immediately cover each well with a drop of fluorescent conjugate. DO NOT LEAVE WELLS UNCOVERED FOR LONGER THAN 15 SECONDS. Drying out of the substrate seriously affects the results.
   The use of a monkey adsorbed conjugate will greatly enhance results (eg FA003.M).
7. Slide Incubation
   Incubate slides for 30 minutes in humid chamber at room temperature (18°C-28°C), in the dark.
8. PBS Wash
   Wash again as described in step 4.
   OPTIONAL COUNTERSTAIN. Add up to 5 drops of 1% Evans Blue per 100mL of PBS prior to slide immersion.
9. Mounting with coverslip
   Remove one slide at a time from PBS wash. Quickly dry around the wells and add a drop of mounting medium to each well. Carefully lower the slide onto the coverslip, avoiding air bubbles, but if present do not attempt to remove. Wipe excess medium from around edge of coverslip.

Slides may be stored for up to 3 days at 2 - 8°C, in the dark, without significant loss of fluorescence.

8 RESULTS

A negative sample should show dull green staining over the sections, with no discernible fluorescence.

An ANNA-1 positive sample should give apple green staining of most neuronal nuclei in the cerebellum, including the purkinje cells. Myenteric plexus neurone nuclei are positive with ANNA-1 (anti-Hu) but negative with ANNA-2 (anti-Ri). There should be no discernible fluorescence in the liver with ANNA-1 positive samples.

A PCA-1 positive sample should give apple green coarse granular staining of the purkinje cell cytoplasm. There should be no discernible fluorescence in the liver with PCA-1 positive samples.

If the controls do not appear as described, the test is invalid and should be repeated.

9 LIMITATIONS OF PROCEDURE

1. The light source, filters and optics of different makes of fluorescence microscopes will influence the sensitivity of the assay. The performance of the microscope is significantly influenced by correct maintenance especially centering of the mercury vapour lamp and changing of the lamp after the recommended period of time.

2. Suitability for use with other manufacturers’ IFA reagents has not been assessed but use with such reagents should not necessarily be excluded.

10 SUMMARY OF PROCEDURE

1. Equilibrate mounting medium to room temperature.
2. Dilute PBS with distilled water.
3. Equilibrate substrate slides to room temperature (18-28°C).
4. Dilute patient sera 1/50.
5. Apply 50µL positive and negative controls and patient sera to appropriate wells.
6. Incubate in a humid chamber for 30 minutes.
7. Wash for 5-10 minutes in PBS.
8. Blot around each well and immediately cover each well with a drop of conjugate.
9. Incubate as in step 5.
10. Wash as step 6.
11. Mount.
12. View slide under fluorescence microscope.

THE BINDING SITE LIMITED

PO Box 4073
Birmingham
B29 6AT
England