FITC IgG (H&L) Conjugate
For In Vitro Diagnostic Use

Product Code: 504032

Intended Use
This product is intended for use in direct and indirect immunofluorescence for the detection of tissue antigens and autoantibodies.

Principles of the Procedure
When used in a direct immunofluorescence technique, the conjugate is diluted appropriately and incubated with substrate tissue. Unbound conjugate is washed off and slides are viewed with a fluorescence microscope. When used in an indirect immunofluorescence technique, the patient’s samples and appropriate controls are incubated with substrate tissue. Unbound antibodies are washed off and appropriately diluted conjugate is applied. Unbound conjugate is washed off and slides are viewed with a fluorescence microscope. With both techniques, positive samples are observed as apple green fluorescence corresponding to the antibody of interest.

Reagent
Presentation
Affinity purified sheep immunoglobulin bound to fluorescein isothiocyanate (FITC) diluted in phosphate buffered saline pH 7.2 (containing 1% bovine serum albumin, 0.099% sodium azide).

Immunogen
Human IgG purified from human serum.

Preparation
Antiserum is prepared by immunising sheep with the above immunogen. The resulting antiserum is adsorbed to monospecificity and then affinity purified. Cross reactivity with bovine, mouse and rat serum proteins is minimised by further insoluble adsorption. The antiserum is then conjugated with FITC. Unreacted fluorochrome is removed by gel filtration. Preservatives are added and the product 0.2μm filtered.

Specificity
The unconjugated immunoglobulin was shown to be specific by immunoelectrophoresis and double diffusion (Ouchterlony). Cross reactivity with bovine, mouse and rat serum proteins has been minimised by insoluble adsorption.
This product has been shown to give appropriate reactivity against a wide range of autoantibodies.

Warnings/Precautions
This product should only be used by suitably trained persons for the purposes stated. This product contains sodium azide and must be handled with caution – do not ingest or allow contact with the skin or mucous membranes. If contact does occur wash with a large volume of water and seek medical advice. Explosive metal azides may be formed with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.
Storage Conditions
Upon receipt the product should be stored at 2-8°C where it will remain stable until the given expiry date. FITC conjugates should be kept out of sunlight, fluorescent and U.V. light whenever possible. Slight precipitation can occur on storage, which may be removed by centrifugation, and should not affect performance characteristics.

Prepare conjugate dilutions immediately prior to use.

Specimen Collection and Preparation
For direct methods freshly frozen tissue should be used. The use of sub-optimally collected and prepared specimens may result in poor quality staining.

For indirect methods 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 7 days prior to assay, or for prolonged storage, aliquoted and stored at -20°C or below. Repeated freezing and thawing should be avoided. Avoid using lipaemic, haemolysed or microbially contaminated sera as decreased titres or unclear staining patterns may occur.

Procedure
Materials provided
- 1.0 mL FITC IgG (H&L) Conjugate
- Product Insert

Additional Materials Required But Not Provided
1. Substrate slide
2. 0.2M TRIS buffered saline pH 7.4 (TBS) for tissues or 0.1M phosphate buffered saline (PBS) for cells
3. Humidity chamber for incubation steps
4. Suitable negative and positive control sera
5. Mounting media and coverslips
6. Fluorescence microscope with 495nm exciter filter and 515nm barrier filter

1 and 4 are not required for direct immunofluorescence.

Product Use
This product has been evaluated by indirect immunofluorescence against the substrates listed below. The dilution ranges recommended were effective in demonstrating known autoimmune sera. The titres reported should be used as guidelines for determining the working dilution in the users system.

RECOMMENDED DILUTIONS OF CONJUGATE

<table>
<thead>
<tr>
<th>SUBSTRATE</th>
<th>INOVA Diagnostics PRODUCT CODE</th>
<th>CONJUGATE DILUTION RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEp2 cells</td>
<td>508100</td>
<td>1/100 – 1/200</td>
</tr>
<tr>
<td>Crithidia luciliae</td>
<td>508200</td>
<td>1/400 – 1/800</td>
</tr>
<tr>
<td>Human Neutrophils</td>
<td>508290/508296</td>
<td>1/400 – 1/800</td>
</tr>
<tr>
<td>Rat/Mouse liver,kidney, stomach</td>
<td>504180/504170</td>
<td>1/50 – 1/100</td>
</tr>
<tr>
<td>Monkey, oesophagus</td>
<td>504145/504150</td>
<td>1/50-1/100</td>
</tr>
</tbody>
</table>
Results
A positive specimen or control should give specific apple green fluorescence.$^1$
A negative specimen or control should show no discernible fluorescence.

Limitations of the Procedure
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 centring of the mercury vapour lamp and changing of the lamp after the recommended period of time.

This reagent is used to aid diagnosis only. A positive result suggests certain diseases which must be confirmed by clinical findings and other serological tests.

References

Manufactured By:
INOVA Diagnostics, Inc.
9900 Old Grove Road
San Diego, CA 92131
United States of America
Technical Service (U.S. & Canada Only) : 877-829-4745
Technical Service (Outside the U.S.) : 00+ 1 858-805-7950
support@inovadx.com

Authorized Representative in the EU:
Medical Technology Promedt Consulting GmbH
Altenhofstrasse 80
D-66386 St. Ingbert, Germany
Tel.: +49-6894-581020
Fax.: +49-6894-581021
www.mt-procons.com

624032ENG August 2011 Revision 0