**FITC IgG Conjugate**  
*For In Vitro Diagnostic Use*

**Product Code: 504033**

**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 sample 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 goat immunoglobulin bound to fluorescein isothiocyanate (FITC) diluted in phosphate buffered saline pH 7.6 (containing 15mg/mL bovine serum albumin, 0.05% sodium azide).

**Immunogen**  
Human IgG (Fc) fragment specific.

**Preparation**  
Antiserum is prepared by immunizing goats with human IgG, Fc fragment purified from normal human serum. The resulting antiserum is delipidated. Affinity purified antibodies are isolated by binding to human IgG attached to agarose beads, followed by a proprietary elution process. The purified antibody is then conjugated with fluorescein isothiocyanate (FITC) and unreacted fluorochrome is removed by gel filtration. This product is diluted to an optimal concentration for use in indirect immunofluorescence. Preservatives are added and the product 0.2µm filtered.

**Specificity**  
Based on immunoelectrophoresis and/or ELISA, the antibody reacts with the Fc portion of human IgG heavy chain but not with the Fab portion of human IgG. No antibody was detected against IgM, IgA or against non-immunoglobulin serum proteins. The antibody may cross-react with immunoglobulins from other species.

**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 (ref. ²), 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.

**Procedure**

**Materials provided**
- 1.0 mL FITC IgG Conjugate
- Product Insert
Additional Materials Required But Not Provided
1. Substrate slide
2. 0.2M TRIS buffered saline pH7.4 (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>
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<tbody>
<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>1/800 – 1/1600</td>
</tr>
<tr>
<td>Rat/Mouse liver, kidney, stomach</td>
<td>504170/504180</td>
<td>1/50 – 1/100</td>
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<tr>
<td>Monkey oesophagus</td>
<td>504145/504150</td>
<td>1/50 – 1/100</td>
</tr>
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Results
A positive specimen or control should give specific apple green fluorescence. 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