Gene Biosolution

FLUORESCEIN CONJUGATED ANTISERUM TO:

HUMAN IgA F/P 2-4 Product #102202

FOR
LABORATORY
USE ONLY

(A) CHARACTERISTICS

I. Description

Antiserum to human **IgA** is produced in goats by immunization with purified human **IgA**. The gamma globulin fraction of the absorbed, monospecific (alpha chain) antiserum is then prepared, followed by reaction with fluorescein isothiocyanate. The excess dye is removed by dialysis and the conjugated globulin further fractionated on DEAE cellulose to remove under- and overlabelled protein. Total protein is determined by the Biuret reaction and the concentration of fluorescein by comparison with a fluorescein diacetate standard. Sodium azide (0.1% w/v) is added as preservative.

A fluorescein to protein ratio of less than 5 is best suited for direct fluorescence testing of tissues.
For indirect fluorescence or for cell suspensions such as lymphocytes catalog No. 102302 with F/P ratios greater than 5 ugF/mgProtein is recommended. Pertinent data for the enclosed antiserum is shown below.

Specificity: Human **IgA** (alpha chain)

Fluorescein:	**76** mcg/ml	**Antibody:**	**5.5** mgAb/ml
Protein:	**19** mg/ml	**Lot No.:**	**1022MI2**
F/P ratio:	**4** mcg/mg	**Expiration :**	**2019-02-21**

At 0° C or below

II. Storage and Handling:

Fluorescein conjugated antisera are stable for 2 years when stored frozen. Repeated freezing and thawing will cause loss of antibody activity and may release free fluorescein dye. Thus, if extended
storage is anticipated the conjugate should be aliquoted and frozen. At refrigerator temperatures, the conjugates are stable for at least 3 months. Exposure to light is detrimental to the material. After thawing, the conjugate should be thoroughly , but gently mixed.

Titration of the conjugate is recommended for each laboratory's application. Dilution to the working titer should be performed for each use and the unused portion discarded after one day. The most commonly employed diluents are saline (0.15**M**), or phosphate-buffered saline (u=.15, pH 7.0-7.4) with or without 4% (w/v) bovine serum albumin (Fraction V) as a stabilizing agent.

(B) LIMITATIONS

I. General

Three types of fluorescence may occur when examining specimens in various test systems.

Nonspecific fluorescence may be due to the natural **autofluorescence** of some tissues and is seen when no conjugated antiserum is present in the test system. Another type of nonspecific fluorescence
is due to the nonimmunological adherence of free fluorescein, aggregated conjugate or tagged non-immunoglobulin (especially albumin) to the substrate. The latter is essentially eliminated by DEAE cellulose fractionation of Gene Biosolution conjugates.

Unwanted specific fluorescence is due to the presence of contaminating antibody in the preparation. It is usually due to cross

reactions between species, such as that seen when anti human IgG reacts with rabbit IgG. Suitable controls should be employed for each system to rule out such activity.
Specific fluorescence is due to the antigen-fluorescent antibody reaction between **IgA** and anti-**IgA**. The specific antibody level is generally greater than 10% of the total protein and the working titer is sufficiently high to eliminate both nonspecific and unwanted specific fluorescence.

II. Loss of free fluorescein from the antibody during storage may cause nonspecific staining. The presence of free dye can be detected by the presence of an anodic band of fluorescence following electrophoresis on cellulose acetate and exposure to ultraviolet light. The free dye may be removed by dialysis against phosphate buffered saline (0.1**M** pH 7-7.4).

III. Aggregation of the conjugated protein may also cause nonspecific fluorescence. These may be removed by centrifugation (1,000 x G) or filtration (0.22 micron).

IV. Microbial contamination may also occur in spite of the preservative if nonsterile pipettes, etc., are used to remove the product from the vials. Characteristic odors or sediments usually indicate contamination, and the conjugate should be discarded. If refiltered, retitration is mandatory.

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