Description **Fluorescein isothiocyanate–conjugated IgG fraction of polyclonal rabbit antiserum to mouse IgG, IgA and IgM, heavy and light chains**

Product code RAM/Ig/FITC

Biological origin Rabbit

Physical form FITC-coupled purified hyperimmune rabbit IgG lyophilized from a solution in phosphate buffered saline (PBS, pH 7.2).

Preservative No preservative added, as it may interfere with the antibody activity.

Immunogen Purified polyclonal mouse IgG, and homogenous IgA and IgM isolated from mouse serum. Freund's complete adjuvant is used in the first step of the immunization procedure.

Purification The IgG (7S) fraction is isolated and purified from hyperimmune antisera with strong precipitating activity and contains the bulk of the antibody specificity. It is free of other serum proteins as tested by immunoelectrophoresis and double radial immunodiffusion.

Adsorption Immunoaffinity adsorbed using insolubilized antigens as required to eliminate antibodies cross-reacting with other serum proteins. The use of insolubilized adsorption antigens prevents the presence of excess adsorbent protein or immune complexes in the antiserum.

Identity & Specificity The reactivity of the antiserum is directed to the major isotypes of the mouse immunoglobulin system (classes and both light chain types) including antibodies to common determinants, to class and to the surface determinants of the common Fab portion, as tested by immunoelectrophoresis and double radial immunodiffusion against pooled serum and purified immunoglobulins. In immunoelectrophoresis and double radial immunodiffusion using various antiserum concentrations against normal mouse plasma and serum, the characteristic IgG, IgA and IgM precipitin lines are obtained.

Cross-reactivity Inter-species cross-reactivity is a normal feature of antibodies to mammalian immunoglobulins, since homologous proteins of different species frequently share antigenic determinants. The degree of cross-reactivity is also dependent on the concentrations of the reactants and the sensitivity of the assay arrangement. This antiserum fraction has been tested for cross-reactivity by double radial immunodiffusion against several species sera with the following results:

bovine +	duck ±	horse +	rat ++
cat +	goat +	human +	sheep +
chicken -	guinea pig ++	monkey +	swine +
dog +	hamster ++	pigeon ±	turkey ±

A negative cross-reaction in double radial immunodiffusion does not exclude some reaction in more sensitive techniques.

Physicochemical characteristics IgG protein concentration 8.3 mg/ml. Fluorochrome/IgG protein molar ratio (F/P) is approximately 1.7. No foreign proteins added.

Fluorescent marker Fluorescein isothiocyanate isomer 1 (FITC). Excitation: 492 nm, emission : 515 nm.

Conjugation procedure A proprietary technique for the binding to FITC is used, followed by several purification steps to remove free reactants and protein aggregates. After each step activity and specificity are tested in a variety of techniques. The conjugate is lyophilized to assure stability and long shelf life.

Intended use Direct immunofluorescence staining of cytoplasmic Ig of appropriately treated cell and tissue substrates; to demonstrate immunoglobulins or specific antibodies in cells and tissues; to identify circulating antibodies in serodiagnostic microbiology and autoimmune diseases; to identify a specific antigen or immune complex using a reference antibody of mouse origin in the middle layer of the indirect test procedure. The presence of activity to the common Ig/Fab subunit may result in the staining of immunoglobulins bound to Fc-receptors on non-lymphoid cells. Combinations of isotype-specific reagents or GAM/Ig(Fc)/FITC should be used instead for this purpose.
This immunoconjugate is not pre-diluted. The optimum working dilution of each conjugate should be established by titration before being used. Excess labelled antibody must be avoided because it may cause high unspecific background staining and interfere with the specific signal.
Working dilutions are usually between 1:40 and 1:120.

Handling The lyophilized conjugate is shipped at ambient temperature and may be stored at +4°C; prolonged storage at or below -20°C. It is reconstituted by adding 1 ml sterile distilled water. Spun down to remove insoluble particles, divided into small aliquots, frozen and stored at or below -20°C. Prior to use, an aliquot is thawed slowly in the dark at ambient temperature, spun down again and used to prepare working dilutions by adding sterile phosphate buffered saline (PBS, pH 7.2). Repeated thawing and freezing should be avoided. Working dilutions should be stored at +4°C, not refrozen, and preferably used the same day. If a slight precipitation occurs upon storage, this should be removed by centrifugation. It will not affect the performance of the immunoconjugate.

Packing Vial with 1 ml lyophilized immunoconjugate.

Storage / shelf life

Lyophilized at +4°C	at least 10 years
reconstituted at or below -20°C	3-5 years
reconstituted at +4°C	7 days

Caution This immunoconjugate should be handled by qualified persons only and appropriate precautions should be taken in its handling and disposal, and of all associated materials. For *in vitro* laboratory research purposes only.

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