Description **Tetramethylrhodamine isothiocyanate–conjugated IgG fraction of polyclonal goat antiserum to mouse Fab of IgG**

Product code GAM/Fab/TRITC

Biological origin Goat

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

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

Immunogen Purified Fab of normal IgG isolated form mouse serum. Freund's complete adjuvant is used in the first step of the immunization procedure.

Purification Hyperimmune antisera with strong precipitating activity are selected for fractionation by salt-precipitation and purification of the IgG fraction by DEAE-chromatography.

Adsorption Immunoaffinity adsorbed using insolubilized antigens as required to eliminate antibody activity to any other serum protein. 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 Fab subunits of intact IgG, IgA IgM and other Ig classes of both light chain types, with their Fab or F(ab')2 subunits, and with free light chains of kappa and lambda type. In immuno-electrophoresis and double radial immunodiffusion using various antiserum concentrations against pooled mouse serum a characteristic precipitin line is obtained, which shows a reaction of identity with the precipitin line of the purified Fab.

Cross-reactivity Inter-species cross-reactivity is a normal feature of antibodies to immunoglobulins, since Ig of different species frequently share antigenic determinants. Cross-reactivity of this antiserum has been tested in double radial immunodiffusion with the following results:

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

Physicochemical characteristics IgG protein concentration 10 mg/ml. Fluorochrome/IgG protein molar ratio (F/P) is approximately 1.7. No foreign proteins added.

Fluorescent marker Tetramethylrhodamine isothiocyanate isomer R. It has an orange-red fluorescence. Excitation: 554 nm, emission: 573 nm. To avoid nonspecific background staining, specially synthesized and exceptionally pure crystalline isomer R has been used instead of the usual racemic mixture. Although its fluorescence efficiency is less than of FITC, TRITC conjugates have the advantage of significantly less photo bleaching. This facilitates their use in quantitative cell-counting procedures.

Conjugation procedure A proprietary technique for the binding to TRITC 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 staining of fixed cell and tissue substrates; to demonstrate the intracellular presence of free or Ig-bound subunits of both kappa or lambda type.
In general this conjugate is not recommended as direct or indirect screening reagent for If isotypes on surface membranes of vital lymphoid cells. The activity to the common Ig/Fab subunit may result in the staining of immunoglobulins bound to the Fc-receptors on non-lymphoid cells. Combinations of isotype-specific reagents 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 1:20 and 1:80.

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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