Description	**Tetramethylrhodamine isothiocyanate-conjugated IgG fraction of polyclonal sheep antiserum to mouse IgG2a, subclass specific**
Product code	ShAM/IgG2a/TRITC
Biological origin	Sheep
Physical form	TRITC-coupled purified hyperimmune sheep 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	Pools of purified homogenous IgG2a isolated from mouse serum of strains belonging to the allelic types Igh-1a and Igh-1b. 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 and purification of the IgG (7S) fraction containing the bulk of the defined antibody specificity. It is free of other serum proteins as tested by immunoelectrophoresis.
Adsorption	Immunoaffinity adsorbed using insolubilized antigens as required to eliminate antibodies cross-reacting with other components of the immunoglobulin system or reacting with other serum proteins. Special attention is given to the removal of antibodies to common Ig/Fab. 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 subclass IgG2a. It reacts strongly withIg2a of the allelic types Igh-1a and Igh-1b, which include BALB/C, C57BL, CBA/J, SJ/J and SM/J. When used fro the identification of IgG2a in other mouse strains the reaction may be weaker. The antiserum contains antibodies to iso and allospecific determinants. It does not react with other subclasses of IgG, IgG/Fab fragments, IgM and IgA or any non-Ig protein in mouse serum, as tested by immunoelectrophoresis and double radial immunodiffusion.
Cross-reactivity	This immunoconjugate is not species-specific since inter-species cross-reactivity is a normal feature of antisera to immunoglobulins. However this conjugate has been passed over appropriate immuno-adsorbents to remove antibodies cross-reacting with human immunoglobulins. This renders it specific for use in test systems containing material of human origin (e.g. human tissue/mouse monoclonal antibody to a human tissue constituent/anti mouse Ig isotype-specific immunoconjugate.
Physicochemical characteristics	IgG protein concentration 10 mg/ml. Fluorochrome/IgG protein molar ratio (F/P) is approximately 1.9. 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 of 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	In immunocytochemical and immunohistochemical single and double staining for the detection of IgG2a at the cellular and subcellular level by staining of appropriately treated cell and tissue substrates; as indicating second antibody conjugate to analyze or separate human cells after reacting with monoclonal mouse antibodies in immunofluorescence microscopy, fluorescence activated cell sorting and flow cytometry; to identify and measure IgG2a in mouse serum or other body fluids. *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, depending on the method used.
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 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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