

Description	Tetramethylrhodamine isothiocyanate-conjugated IgG fraction of polyclonal goat antiserum to C3c fragment of monkey complement factor C3	
Product code	GAMon/C3c/TRITC	
Biological origin	Goat	
Physical form	Fluorochrome-coupled purified hyperimmune 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	C3c is the major fragment resulting from C3 cleavage by C3 convertase and factor I. It is composed of an intact beta chain bound to two fragments of the alpha chain. C3c is isolated and purified from pooled normal monkey 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 the antiserum and contains the bulk of the defined 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 with other plasma proteins. The use of insolubilized adsorption antigens prevents the presence of excess adsorbent protein or immune complexes in the antiserum.	
Identity & Specificity	In immunoelectrophoresis against fresh monkey serum, a single precipitin line is obtained in the beta-1 region representing native C3. Against serum containing partly activated C3, a precipitin line is obtained which extends from the beta-1 into the alpha-2 region, demonstrating a gradient. In old serum containing totally activated C3 a single precipitin line in the alpha-2 region is obtained. Antisera to C3c can also react with the fragments C3b, C3bi and smaller fragments, since they all carry antigenic determinants of the C3c domain. The product does not react with any other proteins component of monkey serum or plasma.	
Cross-reactivity	The antiserum does not cross-react with any other component of monkey plasma. Inter-species cross-reactivity is a normal feature of antibodies to plasma proteins since they frequently share antigenic determinants. In addition to a clear reactivity with C3c of other old-world monkeys (Cercopithecus, Cynomolgus and Baboon), this antiserum shows also a fair amount of cross-reactivity to C3C of other species, including chimpanzee and man.	
Physicochemical characteristics	IgG protein concentration 10 mg/ml. Fluorescein/IgG protein molar ratio (F/P) approximately 2.0. 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	The fluorescent immunoconjugate to monkey C3c is used to determine the presence and pattern of C3 in tissue lesions using immunohistochemical staining techniques. Locally deposited immune complexes in tissue usually contain complement, pointing to activation of the classical pathway. Complement activation in vivo implies active disease and may contribute to the elicitation of the pathogenesis and the extent of tissue destruction. Sometimes the diagnosis can be based on directly on laboratory findings. 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 1:10 and 1:40.	
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 <i>in vitro</i> research purposes only.	

NORDIC IMMUNOLOGICAL LABORATORIES

Langendijk 25, 5652 AX Eindhoven, The Netherlands
Tel. +31 630 070 625, Fax: +31 402 920 069
E-mail: info@nordiclabs.nl
www.nordiclabs.nl