

Description	Horseradish peroxidase-conjugated IgG fraction of polyclonal goat antiserum to C3c fragment of rat complement factor C3	
Product code	GARa/C3c/PO	
Biological origin	Goat	
Physical form	Peroxidase-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 rat 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 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 rat 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 rat serum or plasma.	
Cross-reactivity	The antiserum does not cross-react with any other component of rat plasma. Inter-species cross-reactivity is a normal feature of antibodies to plasma proteins since they frequently share antigenic determinants. Cross-reactivity of this antiserum has not been tested in detail.	
Physicochemical characteristics	IgG protein concentration 10 mg/ml. Peroxidase/IgG protein molar ratio (E/P) approximately 1.8. No foreign proteins added.	
Enzyme marker	Horseradish peroxidase enriched for isoenzyme C (RZ=3.2)	
Manufacturing procedure	A proprietary modification of the periodate technique for the binding of peroxidase is used, followed by several purification steps. 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 enzyme-immunocytochemical and histochemical staining of rat C3c in appropriately prepared substrates at the cellular and subcellular level. 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. In ELISA and Western blotting to identify rat C3c in serum or other body fluids. <i>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.</i> Working dilutions for histochemical and cytochemical use are usually between 1:100 and 1:500; In ELISA and comparable non-precipitating antibody-binding assays between 1:1,000 and 1:5,000.	
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 antiserum 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.	

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