

Description	Soluble rabbit peroxidase-anti-peroxidase immune complex	
Product code	R/PAP	
Biological origin	Rabbit	
Physical form	Hyperimmune rabbit IgG against horseradish peroxidase reacted with peroxidase lyophilized from a solution in phosphate buffered saline (PBS, pH 7.2).	
Preservative	No preservative added, as it may interfere with the enzyme activity.	
Immunogen	Highly purified horseradish peroxidase.	
Physicochemical characteristics	IgG protein concentration 10 mg/ml. No foreign proteins added.	
Intended use	Peroxidase staining of unlabelled rabbit IgG antibodies.	
Staining procedure	<p>All steps are performed at room temperature.</p> <ol style="list-style-type: none">1 Cover tissue sections with normal serum from the same animal species that produced the secondary antibody (step 4), undiluted or in a dilution with PBS up to 1: 20. Incubate 10 minutes and drain. Wash once with PBS.2 Cover section with primary rabbit antiserum diluted in PBS and incubate 30-60 minutes. It may be necessary to incubate overnight in a moist chamber if tissue antigen is limiting or if greater penetration is required to increase antigen detection signal. Drain and wash gently by flooding section with PBS. Incubate 5-10 minutes. Repeat wash two times at 5-10 minutes intervals.3 Repeat the first step if persistent background is encountered in preliminary trials.4 Cover tissues section for 30-60 minutes with secondary anti rabbit IgG (H+L) antiserum, diluted with PBS to 1:50 to 1:100. The secondary antiserum is used in excess to ensure free antigen-binding sites to bind the PAP complex. Drain and wash gently by flooding section with PBS. Incubate 5-10 minutes and drain. Repeat wash two times at 5-10 minutes intervals.5 Make a solution of the R/PAP of 25-50 µg/ml with PBS containing 2% normal serum of the same host as the secondary antiserum. Incubate 30-90 minutes, drain and wash gently by flooding section with PBS. Incubate 5-10 minutes and drain. Repeat wash two times at 5-10 minutes intervals.6 Cover section with 0.05 M Tris-HCl, pH 7.6 containing 0.015% H₂O₂ and 0.05% diaminobezidinetetrahydrochloride. Incubate for 30-60 minutes, dehydrate and mount for microscopy. <p>If residual endogenous peroxidase activity is encountered, cover tissue section with 0.5-3.0% H₂O₂ in absolute methanol for 5 minutes, drain off H₂O₂ and gently flood section with PBS. Incubate 5 minutes, drain and repeat with fresh buffer.</p>	
Handling	<p>The lyophilized product 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 product.</p>	
Packing	Vial with 1 ml lyophilized PAP complex.	
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 product 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> laboratory research purposes only.	

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