2 Steps HRP Detection System			REF	Volume
	Availability	4 Components	PSW-DKI1002K-0150 PSW-DKI1002K-0550 PSW-DKI1002K-1100	15 ml - 150 Test 55 ml - 550 Test 110 ml - 1100 Test
		5 Components	PSW-DKI1002X-0550 PSW-DKI1002X-1100	55 ml - 550 Test 110 ml - 1100 Test

Intended use

PathoSage 2 Steps HRP Detection System Peroxidase Goat Anti-Mouse/Rabbit IgG HRP with DAB, is intended for use in immunohistochemistry for the detection of mouse or rabbit antibodies.

Specification

PathoSage 2 Steps HRP Detection System is good for low antigen expression levels and primary antibody dilution maximization. They consist of an amplifier antibody followed by an affinity-purified and cross-adsorbed polymer reagent to ensure high sensitivity and low background.
The PathoSage 5 Component Detection Kit includes Peroxidase solution. Peroxidazed blocking solution is a highly stable form of hydrogen peroxide for blocking endogenous peroxidase. It is very effective for blocking non-specific staining in red blood cells. It is non-flammable, safer and less toxic when compared to hydrogen peroxide/methanol formulations.
PathoSage 2 steps HRP detection system, peroxidase Goat Anti-Mouse/Rabbit HRP plus DAB, is a Ready-to-Use system that has been manufactured to give an optimal staining when using the protocol advised in this IFU. Prior to staining, some Formalin Fixed, Paraffin-Embedded (FFPE) tissue sections should be subjected to pre-treatment (HIER or digestive enzyme). The PathoSage detection system detects Mouse or Rabbit antibodies bound to an antigen in tissue sections. The antibodies are not provided, but it is recommended to use the PathoSage antibodies.
The polymer-complex is then visualized with a suitable substrate/chromogen. The clinical interpretation of any staining or its absence should be determined by a qualified pathologist and complemented by morphologic studies; controls should be evaluated within the context of the patient's clinical history and/or other diagnostic tests.

Principle of Method

Step	Reagent	Template step	Incubation time
1	Deparaffinize and rehydrate tissue section	Slide / tissue preparing	-
2	Wash Aqua dest	Wash	2x 5 min
3	If applicable; HIER or digestive enzyme	Pre-treatment	*
4	Wash buffer	PBS or TBS buffer	2x 5 min
5	H_2O_2 (concentrate 3%)	Tissue preparing	10 min
6	Peroxidase solution**	Peroxidase Block	10 min
7	Wash buffer	PBS or TBS buffer	2x 5 min
8	Primary mouse or rabbit antibody	Antibody	30 min
9	Wash buffer	PBS or TBS buffer	2x 5 min
10	Detection system, post-blocking	Post-blocking	15 min
11	Wash buffer	PBS or TBS buffer	2x 5 min
12	Detection system, polymer Mouse/Rabbit HRP	Labeled polymer	30 min
13	Wash buffer	PBS or TBS buffer	2x 5 min
14	Substrate	DAB see note	8 min
15	Wash aqua dest	Wash	2x 2 min
16	Counterstain, dehydrate and coverslip	Auxiliary	-

*** See applicable IFU**

Note:

Preparation DAB: Add 40µl DAB Solution B (one drop) to 1 ml substrate Solution A, mix well. Volume and the quality of the DAB has been formulized so they also can be used in automatic stainers, when a higher volume is required.

**** Only Available in 2 Steps HRP 5 Components Kit Detection System**

Materials Provided

REF	Post-Blocking (RTU)	Polymer Goat Anti-Mouse/Rabbit HRP (RTU)	Peroxidase block solution (RTU)	DAB Solution A Buffered H_2O_2 (RTU)	DAB Solution B (Conc.)
PSW-DKI1002K-0150	15 ml	15 ml	-	20 ml	1 ml
PSW-DKI1002K-0550	55 ml	55 ml	-	83 ml	4 ml
PSW-DKI1002K-1100	110 ml	110 ml	-	165 ml	7.5 ml
PSW-DKI1002X-0550	55 ml	55 ml	55 ml	83 ml	4 ml
PSW-DKI1002X-1100	110 ml	110 ml	110 ml	165 ml	7.5 ml

Materials Required but not Provided

Positive and negative control tissues
Primary antibody or antibody cocktail
Primary antibody diluent
Ethanol, distilled or deionised H2O
Wash buffer (PBS or TBS)
Xylene or suitable substitutes
IHC Pen
Negative control reagent
Reagents for enzyme digestion or heat pre-treatment
Chromogenic substrate
Counterstain solution
Mounting medium
Cover slips

Storage and Stability

The solutions should be stored at 2-8°C without further dilution. Please store the reagents in a dark place and do not freeze them. Under these conditions the solutions are stable up to the expiry date indicated on the label. They should not be used after the expiry date.
A positive and a negative control have to be carried out in parallel to the test material. If you observe unusual staining or other deviations from the expected results which could possibly be caused by the kit reagents, please contact PathoSage technical support or your local distributor.

Warnings and Precautions

Use by qualified personnel only. Wear protective clothing to avoid eye, skin or mucous membrane contact with the reagents. In case of a reagent coming into contact with a sensitive area, wash the area with large amounts of water. Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appear. Material Safety Data Sheets (MSDS) are available upon request.

General Limitations

Immunohistochemistry is a complex method in which histological as well as immunological detection methods are combined. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results. The reagent system is especially developed for double color staining with pairs of primary antibodies. One antibody has to be from mouse, one from rabbit. Primary antibodies from other species have to be detected via different detection systems. The same applies for two primary mouse antibodies or two primary rabbit antibodies. It is essential to check if both antibodies are compatible with the same epitope retrieval technique. Endogenous peroxidase or pseudoperoxidase activity may cause non-specific staining. The enzyme activity is blocked by incubation with hydrogen peroxide solution. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems.
Inadequate counterstaining and mounting can influence the interpretation of the results. The color intensity of the reaction product can decrease with time, especially when exposed to light.
PathoSage guarantees that the product will meet all requirements described from its shipping date until its expiry date, as long as the product is correctly stored and utilized. No additional guarantees can be given. Under no circumstances shall PathoSage be liable for any damages arising out of the use of the reagent provided.

LABEL AND BOX SYMBOLS

Explanation of the symbols of the product label and box:

	Expiration date		Refer to the instraction of use	REF	Refrence number
	Temperature limit	IVD	Medical product for in vitro diagnosis	LOT	Lot number
	Hazard warning	RUO	For Research use only	CE	A Conformité Européenne