

ImmPRESS® Duet Double Staining Polymer Kit Peroxidase/Alkaline Phosphatase (HRP Anti-Mouse IgG/AP Anti-Rabbit IgG)

Cat. No.: MP-7724

Storage: 2-8 °C

Instructions for Immunohistochemical staining using rabbit and mouse primary antibodies.

DESCRIPTION

The ImmPRESS Duet Double Staining Polymer Kit is an enzymatic, non-biotin amplification system that produces crisp, highly sensitive, specific staining with low background of two different target antigens on the same tissue section. Anti-Mouse and anti-rabbit IgG purified secondary antibodies are independently conjugated with horseradish peroxidase (HRP) and alkaline phosphatase (AP) enzyme, then blended at optimized dilutions to provide a stable pre-diluted, ready-to-use HRP/AP ImmPRESS polymer formulation.

The ImmPRESS Duet Double Staining Polymer Kit is intended for use on non-rodent tissue specimens. Reliable and reproducible results are obtained on tissue sections where the two target antigens do not over-lap (co-localize) in the same structure of the same cell, but rather are expressed in different cell compartments or different cell types.

KIT COMPONENTS

<u>Product Name</u>	<u>Volume</u>
BLOXALL® Blocking Solution	15 ml
Normal Horse Serum, 2.5%	15 ml
ImmPRESS Duet Reagent (HRP Horse Anti-Mouse IgG/AP Horse Anti-Rabbit IgG)	15 ml
ImmPACT DAB EqV Reagent 1 (Chromogen)	7.5 ml
ImmPACT DAB EqV Reagent 2 (Diluent)	7.5 ml
ImmPACT Vector Red Diluent	15 ml
ImmPACT Vector Red Reagent 1	240 µl
ImmPACT Vector Red Reagent 2	180 µl

The ImmPRESS Duet Double Staining Polymer Kit will stain approximately 150 sections.

STORAGE:

- Store reagents in original bottles at 2-8 °C
- Do not freeze.

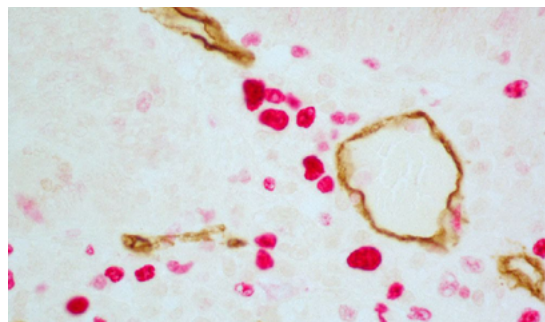
SUBSTRATE PREPARATION

ImmPACT DAB EqV

- Mix equal volumes of Reagent 1 and Reagent 2.
- Use immediately.

ImmPACT Vector Red

- To 2.5 ml of ImmPACT Vector Red Diluent:
- add 40 µl of ImmPACT Vector Red Reagent 1
- add 30 µl of ImmPACT Vector Red Reagent 2
- Use immediately.



Colon Carcinoma (paraffin): ImmPRESS Duet Double Labeling Polymer Kit used to detect mouse anti-CD34 (ImmPACT DAB, brown) and rabbit anti-Ki67 (ImmPACT® Vector® Red, magenta).

STAINING PROCEDURE

1. For paraffin sections, deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
For frozen sections or cell preparations fix with acetone or an appropriate fixative for the antigen under study, if necessary.
Wash for 5 minutes in tap water.
2. If antigen unmasking is required, perform this procedure using a Vector Antigen Unmasking Solution, Citrate-based, pH 6.0 (H-3300) or Tris-based, pH 9.0 (H-3301).
3. If quenching of endogenous peroxidase activity is required, incubate the sections in BLOXALL Blocking Solution for 10 minutes.
4. Wash in buffer for 5 minutes.
5. Incubate sections for 20 minutes with Normal Horse Serum, 2.5%.
6. Tip off excess serum from sections and incubate with rabbit and mouse primary antibodies diluted in appropriate antibody diluent solution, such as diluted normal horse serum or BSA.
7. Wash in buffer for 5 minutes.
8. Incubate for 10 minutes with ImmPRESS Duet Reagent.
9. Wash in buffer for 2 x 5 minutes.
10. Apply ImmPACT DAB EqV Substrate for 2-10 minutes or until desired stain intensity develops. (See "Substrate Preparation")
11. Wash for 2 x 5 minutes in buffer.
12. Apply ImmPACT Vector Red Substrate for 20-30 minutes or until desired stain intensity develops. (See "Substrate Preparation")
13. Rinse slides with buffer.
14. Wash slides in water for 5 minutes.
15. Counterstain (optional), clear and mount.

Detailed product listings, specifications, protocols and additional information are available on our website: vectorlabs.com