



ImmPRESS™ *Duet* Double Staining Polymer Kit Peroxidase/Alkaline Phosphatase

HRP Anti-Rabbit IgG/AP Anti-Mouse IgG

Includes Substrates:

HRP ImmPACT™ DAB EqV (brown)
AP ImmPACT™ Vector® Red (magenta)

Cat. No. MP-7714

Introduction

The ImmPRESS™ *Duet* Double Staining Polymer Kit enables fast, well-defined localization and visualization of two different target antigens on the same tissue section. Utilizing our unique ImmPRESS™ Polymer chemistry, we independently conjugate highly-active horseradish peroxidase (HRP) and alkaline phosphatase (AP) enzyme to kit specific anti-mouse IGG and anti-rabbit IgG affinity purified secondary antibodies. These reagents are then blended at optimized dilutions to provide a stable pre-diluted, ready-to-use HRP/AP formulation that will detect mouse and rabbit primary antibodies, and facilitate a time-saving, simplified, one-step double label solution. For maximum sensitivity, and staining contrast between target antigens, ImmPACT™ DAB EqV (HRP, brown) and ImmPACT™ Vector® Red (AP, magenta) substrates are included to provide a complete easy to use staining kit.

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.

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COMPONENTS

Reagents supplied:

- **BLOXALL™** Endogenous Enzyme Blocking Solution
- 2.5% Normal Horse Serum (ready-to-use)
- **ImmPRESS™ *Duet*** Reagent (mixture of ImmPRESS™-HRP Horse anti-Rabbit IgG and ImmPRESS™-AP Horse anti-Mouse IgG)
- **ImmPACT™ DAB EqV** (HRP, brown)
 - Reagent 1 (Chromogen)
 - Reagent 2 (Buffer)
- **ImmPACT™ Vector® Red** (AP, magenta)
 - Diluent
 - Reagent 1
 - Reagent 2

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

Unless labeled otherwise, ImmPRESS™ *Duet* Double Staining Polymer Kit reagents are designed for laboratory use only.

Storage:

Store ImmPRESS™ *Duet* Double Staining Polymer Kit at 2-8 °C (do not freeze).

Reagents not supplied:

- Buffer
- Primary Antibody/Primary Antibody Diluent
- Counterstain (optional)

ImmPRESS™ *Duet* DETECTION SYSTEM

The ImmPRESS™ *Duet* Double Staining Polymer Kit reagents are ready-to-use — No mixing or titering of the ImmPRESS™ reagent is necessary to obtain optimal immunohistochemical staining. Dilution of this reagent or changes in suggested incubation time may affect performance. The reagent is supplied in a convenient dropper bottle. (To remove the drop dispenser tip, press laterally with thumb until tip snaps off).

The staining procedure should be performed at room temperature (20 - 25 °C). For optimal performance, the ImmPRESS™ Reagent should be equilibrated to room temperature before use.

A number of different wash buffers can be used with the ImmPRESS™ Reagents. One of the most common is 10 mM sodium phosphate, pH 7.5, 0.9% saline (PBS). 0.1% Tween 20 detergent may be added to the wash buffer and is especially recommended for use with automated stainers.

REAGENT 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 40ul of ImmPACT™ Vector® Red Reagent 1
 - add 30ul of ImmPACT™ Vector® Red Reagent 2
- Use immediately.

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 slides for 5 minutes in buffer.
5. Incubate slides with ready-to-use (2.5%) normal horse serum for 20 minutes.
6. Blot excess serum from slides and apply anti-mouse and anti-rabbit primary antibodies diluted in an appropriate antibody diluent solution. *See note 7*
7. Wash slides with buffer for 5 minutes.
8. Incubate slides with ImmPRESS™ *Duet* Detection Reagent for 10 minutes. *See Note 1*
9. Wash slides in buffer 2 x 5 minutes.
10. Apply ImmPACT™ DAB EqV substrate for 2-10 minutes or until suitable staining develops. (See reagent preparation instructions below).
11. Wash slides in buffer 2 x 5 minutes.

- Apply ImmPACT™ Vector® Red substrate for 20-30 minutes or until suitable staining develops. (See reagent preparation instructions below).
- Rinse slides with buffer.
- Wash slides in water for 5 minutes.
- Counterstain slides if desired. Slides can be mounted aqueously using Vectamount AQ (H-5501), or slides can be dehydrated, cleared, and mounted with a non-aqueous media such as Vectamount (H-5000)

NOTES:

- Sensitivity may be increased by lengthening the incubation time of the ImmPRESS™ *Duet* Detection Reagent up to 30 minutes.
- For thicker sections, longer incubation times may be required for optimal staining. Appropriate control slides should be run in parallel if incubation times are altered.
- Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining. It is recommended that enzyme substrate solutions be prepared with glass distilled water. Deionized water (even with low conductivities) may contain enzyme inhibitors of peroxidase and can reduce sensitivity.
- To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.
- To avoid adsorption of the antibody to the plastic or glass container in which the final dilution is made, the primary antibody may be diluted in buffers containing diluted (2.5%) normal horse serum (S-2000 or S-2012). Do not substitute serum from species other than horse.

Alternatively, immunohistochemical grade BSA (SP-5050) can be used. Other grades of BSA can contain undesired impurities.
- Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in preparing the peroxidase detection systems or their substrates. This may result in reduced sensitivity.
- Primary antibodies can be applied separately in sequential incubation steps if preferred.

ImmPRESS™ Reagents:

Alkaline Phosphatase/Peroxidase Duet:

ImmPRESS™ *Duet* Polymer Kit (HRP Anti-Rabbit IgG/AP Anti-Mouse IgG) 1 Kit MP-7714

ImmPRESS™ *Duet* Polymer Kit (HRP Anti-Mouse IgG/AP Anti-Rabbit IgG) 1 Kit MP-7724

Amplified Peroxidase:

ImmPRESS™ *Excel* Reagent Anti-Rabbit IgG 15 ml MP-7601
50 ml MP-7601

ImmPRESS™ *Excel* Reagent Anti-Mouse IgG 15 ml MP-7602
15 ml MP-7602

Peroxidase:

ImmPRESS™ Reagent Anti-Rabbit IgG (made in horse) 15 ml MP-7401
50 ml MP-7401

ImmPRESS™ Reagent Anti-Rabbit IgG (made in goat) 15 ml MP-7451
50 ml MP-7451

ImmPRESS™ Reagent Anti-Mouse IgG (made in horse) 15 ml MP-7402
50 ml MP-7402

ImmPRESS™ Reagent Anti-Mouse IgG (made in goat) 15 ml MP-7452
50 ml MP-7452

ImmPRESS™ Reagent Anti-Mouse IgG (rat adsorbed, made in horse) 15 ml MP-7422

ImmPRESS™ Reagent Anti-Goat IgG (made in horse) 15 ml MP-7405
50 ml MP-7405

ImmPRESS™ Reagent Anti-Rat IgG (made in goat) 50 ml MP-7404

ImmPRESS™ Reagent Anti-Rat IgG (mouse adsorbed, made in goat) 15 ml MP-7444

ImmPRESS™ Universal Reagent Anti-Mouse/Rabbit IgG (made in horse) 15 ml MP-7500
50 ml MP-7500

Vector® M.O.M.™ ImmPRESS™ Peroxidase Kit (made in horse) 1 Kit MP-2400

Veterinary Reagents:

ImmPRESS™ VR Reagent Anti-Rabbit IgG (made in horse) 15 ml MP-6401

ImmPRESS™ VR Reagent Anti-Mouse IgG (made in horse) 15 ml MP-6402

Alkaline Phosphatase:

ImmPRESS™-AP Anti-Rabbit IgG (made in horse) 15 ml MP-5401
50 ml MP-5401

ImmPRESS™-AP Anti-Mouse IgG (made in horse) 15 ml MP-5402
50 ml MP-5402

ImmPRESS™-AP Anti-Goat IgG (made in horse) 15 ml MP-5405
50 ml MP-5405

ImmPRESS™-AP Anti-Rat IgG (made in goat) 15 ml MP-5404

ImmPRESS™-AP Anti-Rat IgG (mouse adsorbed, made in goat) 15 ml MP-5444

Alkaline Phosphatase and Peroxidase Substrates:

All Vector Laboratories' substrates are supplied in convenient, easy to use dropper bottles. Vector Labs offers conventional as well as proprietary substrates producing the colors listed.

Horseradish Peroxidase (HRP):

ImmPACT™ DAB EqV (Brown)	400 ml	SK-4103
ImmPACT™ DAB (Brown)	120 ml	SK-4105
ImmPACT™ AEC (Red)	120 ml	SK-4205
ImmPACT™ AMEC Red (Red)	120 ml	SK-4285
ImmPACT™ VIP (Purple)	120 ml	SK-4605
ImmPACT™ SG (Blue-Gray)	120 ml	SK-4705
ImmPACT™ NovaRED™ (Red)	120 ml	SK-4805
DAB/Ni (Brown or Gray-Black)	1 Kit	SK-4100
AEC (Red)	1 Kit	SK-4200
TMB (Blue)	1 Kit	SK-4400
Vector® VIP (Purple)	1 Kit	SK-4600
Vector® SG (Blue-Gray)	1 Kit	SK-4700
Vector® NovaRED™ (Red)	1 Kit	SK-4800

Alkaline Phosphatase (AP):

ImmPACT™ Vector® Red (magenta)	1 Kit	SK-5105
Vector® Red (magenta)	1 Kit	SK-5100
Vector® Blue (blue)	1 Kit	SK-5300
BCIP/NBT (indigo)	1 Kit	SK-5400
Vector® Black (brown-black)	1 Kit	SK-5200

Note: A chart of the Relative Sensitivity of Substrates in Immunohistochemistry and further descriptions of substrate properties are available on our website.

Other Related Reagents:

Antigen Unmasking Solution (dilutes to 25 liters)		
Citrate-based, pH 6.0	250 ml	H-3300
Tris-based, pH 9.0	250 ml	H-3301
BLOXALL™ Blocking Solution	100 ml	SP-6000
ImmEdge™ Hydrophobic Barrier Pen	2-pen set	H-4000
ImmPrint™ Histology Pen	5-pen set	H-6100
Vectabond™ Reagent (dilutes to 350 ml)	7 ml	SP-1800
VectaMount™ Mounting Medium	60 ml	H-5000
VectaMount™ AQ Mounting Medium	60 ml	H-5501
Vector® Hematoxylin	500 ml	H-3401
Vector® Hematoxylin QS	100 ml	H-3404
Vector® Methyl Green	500 ml	H-3402
Vector® Nuclear Fast Red	500 ml	H-3403
Bovine Serum Albumin (BSA)	500 mg	SP-5050

Heat-treated, ultrafiltered normal serum:

Normal Horse Serum	20 ml	S-2000
2.5% Normal Horse Serum (ready-to-use)	50 ml	S-2012

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Detailed product listings, specifications and protocols are available on our website. A complete catalog listing is also available upon request.

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