**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) enzymes 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 maximum sensitivity, and staining contrast between target antigens. ImmPRESS™ Duet Double Staining Polymer Kit reagents are designed for laboratory use only. 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.

**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

**Staining Procedure**

1. For paraffin sections, deparaffinize and hydrate tissue sections through xylene 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.

**Components**

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

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12. Apply ImmPRESS™ Vector® Red substrate for 20-30 minutes or until suitable staining develops. (See reagent preparation instructions below).

13. Rinse slides with buffer.

14. Wash slides in water for 5 minutes.

15. Counterstain slides if desired. Slides can be mounted aqeuously using Vectamount AQ (H-5501), or slides can be dehydrated, cleared, and mounted with a non-aqueous media such as Vectamount (H-5000)

NOTES:

1. Sensitivity may be increased by lengthening the incubation time of the ImmPRESS™ Duot Detection Reagent up to 30 minutes.

2. 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.

3. 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 conductivity) may contain enzyme inhibitors of peroxidase and can reduce sensitivity.

4. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.

5. 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.

6. 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.

7. Primary antibodies can be applied separately in sequential incubation steps if preferred.

ImmPRESS™ Reagents:

Alkaline Phosphatase/Peroxidase Duet:

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

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

Amplified Peroxidase:

| ImmPRESS™ Exac Reagent Anti-Rabbit IgG | 15 ml | MP-7601 |

| ImmPRESS™ Exac Reagent Anti-Mouse IgG | 15 ml | MP-7602 |

Peroxidase:

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

| ImmPRESS™ Reagent Anti-Mouse IgG (made in goat) | 15 ml | MP-7451 |

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

| ImmPRESS™ Reagent Anti-Rabbit IgG (made in goat) | 50 ml | MP-7452 |

| ImmPRESS™ Reagent Anti-Mouse IgG (made in horse) | 50 ml | MP-7403 |

| ImmPRESS™ Reagent Anti-Goat IgG (made in horse) | 50 ml | MP-7404 |

| ImmPRESS™ Reagent Anti-Rat IgG (made in goat) | 50 ml | MP-7444 |

| ImmPRESS™ Reagent Anti-Rat IgG (mouse adsorbed, made in goat) | 15 ml | MP-7445 |

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

| ImmPRESS™ Exac Polymer® (dilutes to 350 ml) | 7 ml | SP-1800 |

| ImmPRESS™ Exac Polymer® (dilutes to 35 liters) | 120 ml | SK-5200 |

| ImmPRESS™ Universal Reagent Anti-Mouse/Rabbit IgG (made in horse) | 15 ml | MP-7501 |

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.

Horse radish 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™ AEC Red (Red) | 120 ml | SK-4285 |

| ImmPACT™ VIP (Purple) | 120 ml | SK-4605 |

| ImmPACT™ 5G (Blue-Gray) | 120 ml | SK-4705 |

| ImmPACT™ NovaRED® (Red) | 120 ml | SK-4805 |

| DAB/NS (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™ 5G (Blue-Gray) | 1 Kit | SK-4700 |

| Vector™ NovaRED® (Red) | 1 Kit | SK-4800 |

Alkaline Phosphatase (AP):

| ImmPRESS™ 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 | 5-2000 |

| 2.5% Normal Horse Serum (ready-to-use) | 50 ml | 5-2012 |

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