



ImmPRESS™ *Excel* Staining Kit

PEROXIDASE

Anti-Rabbit IgG

Cat. No. MP-7601

Introduction

The ImmPRESS™ *Excel* Amplified Staining Kit is an enzymatic, non-biotin amplification system that produces crisp, highly sensitive, specific staining with low background. This system employs a proprietary ready-to-use (RTU) Amplifier Antibody, followed by a ready-to-use (RTU) ImmPRESS™ *Excel* Polymer Reagent. These reagents are affinity purified and extensively cross-adsorbed to insure high sensitivity and low background. In addition, the ImmPRESS™ *Excel* Reagent is conjugated to horseradish peroxidase “micropolymers”, a novel approach that avoids the use of large dextrans or other macromolecules as a backbone. These unique “micropolymers” allow a higher density of enzymes per antibody to bind the target with minimal interference. The result is an increase in target accessibility, signal intensity, and significantly less background staining.

The ImmPACT™ DAB *EqV* Substrate produces a crisp, dark brown reaction product with the excellent sensitivity and low background characteristic of the ImmPRESS™/ImmPACT™ combination.

COMPONENTS

Reagents supplied:

- BLOXALL™ Endogenous Enzyme Blocking Solution
- 2.5% Normal Horse Serum (ready-to-use)
- Amplifier Antibody (goat anti-rabbit IgG, ready-to-use)
- ImmPRESS™ *Excel* Polymer Reagent (horse anti-goat IgG, ready-to-use)
- ImmPACT™ DAB *EqV* Reagent 1 (Chromogen)
- ImmPACT™ DAB *EqV* Reagent 2 (Buffer)

ImmPRESS™ *Excel* Staining Kit (15 ml) will stain approximately 75-150 sections and an ImmPRESS™ *Excel* Staining Kit (50 ml) will stain approximately 250-500 sections.

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

Storage:

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

Reagents not supplied:

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

ImmPRESS™ DETECTION SYSTEM

The reagents in the ImmPRESS™ *Excel* Staining Kit are ready-to-use — no mixing or titering is necessary to obtain optimal staining. Dilution of the reagents or changes in suggested incubation time may affect performance. The reagents are supplied in convenient dropper bottles. (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™ *Excel* Staining Kit should be at room temperature. Slides should be placed in a humidified chamber during the incubation period.

A number of different wash buffers can be used with the ImmPRESS™ *Excel* Staining Kit. 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.

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 (SP-6000) for 10 minutes. If endogenous peroxidase activity does not present a problem, this step may be omitted.
4. Wash in buffer for 5 minutes.
5. Incubate sections for 20 minutes with 2.5% Normal Horse Serum. Tip off.
6. Incubate with rabbit primary antibody diluted in appropriate antibody diluent solution, such as normal horse serum or BSA. (See Note 4).
7. Wash in buffer for 5 minutes.
8. Incubate for 15 minutes with Amplifier Antibody.
9. Wash in buffer for 5 minutes.
10. Incubate for 30 minutes with ImmPRESS™ *Excel* Reagent.
11. Wash for 2 x 5 minutes in buffer.
12. Combine equal volumes of ImmPACT™ DAB *EqV* Reagent 1 with Reagent 2. Mix well.

Vector Laboratories, Inc.

30 Ingold Road • Burlingame, CA 94010

Tel: (650) 697-3600 • Fax: (650) 697-0339

E-mail: vector@vectorlabs.com • www.vectorlabs.com

13. Incubate in ImmPACT™ DAB EqV working solution until desired stain intensity develops, approximately 2-10 minutes.
14. Wash for 2 x 5 minutes in buffer.
15. Rinse sections in tap water.
16. Counterstain, clear and mount.

Note: A Counterstain/Substrate Compatibility Table is available on our website.

NOTES:

1. 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.
2. 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.
3. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (Cat. No. SP-1800), a non-protein tissue section adhesive.
4. 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 (Cat. No. 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.
5. 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.

ImmPRESS™ Reagents:

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 15 ml MP-7401
(made in horse) 50 ml MP-7401

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

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

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

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

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

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

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

ImmPRESS™ Universal Reagent Anti-Mouse/Rabbit IgG 15 ml MP-7500
(made in horse) 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 15 ml MP-6401
(made in horse)

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

Alkaline Phosphatase:

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

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

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

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

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

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.

ImmPACT™ DAB <i>EqV</i> (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 Substrate (Brown or Gray-Black)	1 Kit	SK-4100
AEC Substrate (Red)	1 Kit	SK-4200
TMB Substrate (Blue)	1 Kit	SK-4400
Vector® VIP Substrate (Purple)	1 Kit	SK-4600
Vector® SG Substrate (Blue-Gray)	1 Kit	SK-4700
Vector® NovaRED™ Substrate (Red)	1 Kit	SK-4800

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

Trademarks: Vector, VECTABOND, BLOXALL, ImmPRESS, ImmEdge, ImmPACT, ImmPrint, VectaMount, Vector NovaRED, and M.O.M. are trademarks of Vector Laboratories.

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

Visit our website: www.vectorlabs.com