



ImmPRESS™ REAGENT Anti-Rat IgG (mouse adsorbed)

PEROXIDASE
Cat. No. MP-7444

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING
USING RAT PRIMARY ANTIBODIES ON MOUSE TISSUE

Introduction

The ImmPRESS™ Polymerized Reporter Enzyme Staining System is an enzymatic, non-biotin, one-step detection kit that provides very high sensitivity with very low background staining in immunohistochemical and immunocytochemical applications.

The ImmPRESS™ Reagent employs a novel, proprietary approach to conjugate horseradish peroxidase micropolymers to our affinity-purified, extensively cross-adsorbed secondary antibodies. This conjugation technology avoids the use of large dextrans or other macromolecules as a backbone and allows a higher density of enzymes per antibody to bind to the target with minimal steric interference. The overall result is an increase in accessibility to the target, binding specificity, and signal intensity with low background staining.

COMPONENTS

Reagents supplied:

- ImmPRESS™ (Peroxidase) Polymer Anti-Rat IgG (mouse adsorbed) Reagent (made in goat, ready-to-use)
- 2.5% Normal Goat Serum for blocking (ready-to-use)

The ImmPRESS™ Anti-Rat Ig (mouse adsorbed) Reagent has $\leq 1\%$ cross-reactivity with mouse IgG and IgM and is designed for use with mouse tissues that may contain mouse immunoglobulins.

The ImmPRESS™ (15 ml) Kit will stain approximately 75-150 sections.

Unless labeled otherwise, ImmPRESS™ reagents are designed for laboratory use only.

Storage:

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

Reagents not supplied:

- Primary Antibody
- Buffer
- Peroxidase Substrate

ImmPRESS™ DETECTION SYSTEM

The ImmPRESS™ Reagent is 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. Adding 0.1% Tween 20 is especially recommended for use with automated stainers.

STAINING PROCEDURE

1. For paraffin sections, deparaffinize and hydrate 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 (H-3300) or High pH-based (H-3301).
3. If quenching of endogenous peroxidase activity is required, incubate the slides in BLOXALL™ Blocking Solution (SP-6000) for 10 minutes. If endogenous peroxidase activity does not present a problem, this step may be omitted. For alternative quenching procedures please see Note 3.
4. Wash in buffer for 5 minutes.
5. Incubate for 20 minutes with ready-to-use (2.5%) normal goat blocking serum or blocking solution of choice. Blot excess serum from sections.
6. Incubate with rat primary antibody diluted in appropriate antibody diluent solution. See Note 4.
7. Wash for 5 minutes in buffer.
8. Incubate for 30 minutes with ImmPRESS™ reagent.
9. Wash for at least 2x 5 minutes in buffer.
10. Incubate in peroxidase substrate solution until desired stain intensity develops. For a list of peroxidase substrates see reverse.
11. Rinse in tap water.
12. Counterstain, clear and mount.

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NOTES:

- ImmPRESS™** reagents are ideal for multiple antigen labeling applications. A brochure is available with protocols - "Discovery Through Color". Please request a free printed copy or download from our website: www.vectorlabs.com. Additional information on Enzyme Substrate Combinations, Counterstain/Substrate Compatibility, and Relative Substrate Sensitivity is also available on our website.
- Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.
- Alternative peroxidase quenching procedures:

For formalin fixed cells and tissues, incubate in 3% H₂O₂ in tap water for 5 minutes or 0.3% H₂O₂ in either methanol or water for 30 minutes.

For frozen tissue or cell preparations, use 0.3% H₂O₂ in 0.3% normal serum in PBS for 5 minutes, or 0.3% H₂O₂ in methanol for 30 minutes or use other published methods. (eg. Andrew, S.M., Jasani, B., Histochem J. 1987, 19, 426-30).
- 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 serum. Alternatively, 0.1% immunohistochemical grade bovine serum albumin (SP-5050) can be used. Other grades of BSA can contain undesired impurities.
- For thicker sections, longer incubation times may be required for optimal staining.
- To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.

Peroxidase Substrates:

A variety of chromogens can be used to localize peroxidase in tissue or cell preparations. All Vector Laboratories' substrates are supplied in convenient, easy to use dropper bottles. Vector Laboratories offers conventional as well as proprietary substrates producing the colors listed.

Note: A chart of the Relative Sensitivity of Substrates in Immunohistochemistry and further description of substrate properties is available on our website: www.vectorlabs.com

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 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

* AEC, ImmPACT™ AEC and ImmPACT™ AMEC Red must be mounted in aqueous mounting media. All other substrates may be dehydrated, cleared, and permanently mounted.

Kits contain sufficient reagent to prepare approximately 300 ml of working solution.

ImmPRESS™ Reagents:

Amplified Peroxidase:

ImmPRESS™ Excel Reagent Anti-Rabbit IgG 15 ml MP-7601
(made in horse)

ImmPRESS™ Excel Reagent Anti-Mouse IgG 15 ml MP-7602
(made in horse)

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

Vector® M.O.M.™ **ImmPRESS™** 1 Kit MP-2400
Peroxidase Kit (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

Related Reagents:

Antigen Unmasking Solution (dilutes to 25 liters)

Citrate-based	250 ml	H-3300
High pH	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

Heat-treated, ultrafiltered normal serum:

Normal Goat Serum	20 ml	S-1000
2.5% Normal Goat Serum	50 ml	S-1012
Normal Horse Serum	20 ml	S-2000
2.5% Normal Horse Serum	50 ml	S-2012
Normal Chicken Serum	20 ml	S-3000
Normal Swine Serum	20 ml	S-4000
Normal Rabbit Serum	20 ml	S-5000

Control Antibodies:

Rabbit IgG Control Antibody	5 mg	I-1000
Mouse IgG Control Antibody	1 mg	I-2000
Rat IgG Control Antibody	1 mg	I-4000
Goat IgG Control Antibody	5 mg	I-5000

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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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