



ImmPRESS™-AP REAGENT Anti-Rat IgG

ALKALINE PHOSPHATASE
Cat. No. MP-5404

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING
USING RAT PRIMARY ANTIBODIES

Introduction

The ImmPRESS™ polymerized reporter enzyme staining system is based on a new method of polymerizing enzymes and attaching these polymers to our affinity-purified, extensively cross-adsorbed secondary antibodies. This approach forms very active enzyme micropolymers, avoiding the use of large dextrans or other macromolecules as a backbone. These unique micropolymers are attached to an antibody allowing a higher density of enzymes to access a target with minimal interference. The ImmPRESS™-AP Reagent has both an optimal size and an ideal enzyme-to-antibody ratio. The overall result is an increase in accessibility to the target, binding specificity, and signal intensity with low background staining.

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COMPONENTS

Reagents supplied:

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

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

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

Storage:

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

Reagents not supplied:

- Primary Antibody
- Buffer
- Alkaline Phosphatase Substrate
- Counterstain (optional)

ImmPRESS™-AP DETECTION SYSTEM

The ImmPRESS™-AP Reagent is ready-to-use — No mixing or titering of the ImmPRESS™-AP 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™-AP Reagent should be equilibrated to room temperature before use.

A number of different wash buffers can be used with the ImmPRESS™-AP 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.

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, pH 6.0 (H-3300) or Tris-based, pH 9.0 (H-3301).
3. Block endogenous enzyme activity. Endogenous alkaline phosphatase activity is less common in paraffin sections than in frozen tissue sections and is generally completely absent in sections treated with high temperature to unmask antigens. For effective and convenient blocking of all forms of alkaline phosphatase, incubate sections with one-step, ready-to-use BLOXALL™ Blocking Solution (SP-6000) for 10 minutes. For alternative blocking protocols see note 3.
4. Wash for 5 minutes in buffer.
5. Incubate for 20 minutes with ready-to-use (2.5%) normal goat serum or blocking solution of choice.
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™-AP Reagent.
9. Wash for 2 x 5 minutes in buffer.
10. Incubate in AP substrate solution until desired stain intensity develops. For a list of AP substrates see reverse.
11. Rinse in tap water.
12. Counterstain, clear and mount.

NOTES:

1. **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.
2. Use only freshly prepared buffers. Bacterial contamination which can occur in buffers stored at room temperature may affect the quality of the staining.
3. Alternative methods for blocking endogenous alkaline phosphatase:
If the endogenous activity is an isoenzyme other than the intestinal form it can be inhibited by adding Levamisole (SP-5000) to the substrate working solution. Intestinal alkaline phosphatase can be inhibited by following procedures described in the following reference: Bulman AS and Heyderman E; J. Clin. Pathol. 34, 1349-1351, 1981.
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 serum. Alternatively, 0.1% immunohistochemical grade bovine serum albumin (SP-5050) can be used. Other grades of BSA can contain undesired impurities.
5. For thicker sections longer incubation times may be required for optimal staining.
6. To prevent sections from detaching from the glass, slides can be treated with VECTABOND™ Reagent (SP-1800), a non-protein tissue section adhesive.

Alkaline Phosphatase Substrates:

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

ImmPRESS™ Reagents:

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	

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	50 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	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 (Peroxidase):

ImmPRESS™ VR Reagent Anti-Rabbit IgG (made in horse)	15 ml MP-6401	
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ImmPRESS™ VR Reagent Anti-Mouse IgG (made in horse)	15 ml MP-6402	
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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

Heat-treated, ultrafiltered normal serum:

Normal Goat Serum	20 ml	S-1000
2.5% Normal Goat Serum (ready-to-use)	50 ml	S-1012
Normal Horse Serum	20 ml	S-2000
2.5% Normal Horse Serum (ready-to-use)	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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