



## ImmPRESS™ REAGENT Anti-Rabbit Ig

**PEROXIDASE**  
Cat. No. MP-7401

INSTRUCTIONS FOR IMMUNOHISTOCHEMICAL STAINING  
USING RABBIT PRIMARY ANTIBODIES

### Introduction

The ImmPRESS™ polymerized reporter enzyme staining system is based on a new method of polymerizing enzymes and attaching these polymers to antibodies. The ImmPRESS™ reagents provide very high sensitivity with very low background staining in immunohistochemical applications. The novel approach employed to form enzyme “micropolymers” avoids the use of large dextrans or other macromolecules as a backbone. Attaching these unique “micropolymers” to an antibody allows a higher density of enzymes to access a target with minimal interference. The result is a reduction in the number of steps in the protocol, an increase in signal intensity, and significantly less background staining.

The ImmPRESS™ anti-rabbit Ig reagent contains a “micropolymer” of a very active peroxidase coupled to our affinity-purified anti-rabbit IgG (H+L) secondary antibody. The ImmPRESS™ reagent is supplied prediluted in a convenient dropper bottle. No mixing or titrating of ImmPRESS™ reagents is necessary to obtain optimal immunohistochemical staining. Ready-to-use 2.5% normal horse serum blocking solution is also included for convenience.

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

### ImmPRESS™ DETECTION SYSTEM

The ImmPRESS™ reagent is supplied as a ready-to-use solution. Dilution of this reagent or changes in suggested incubation time may affect performance. The bottle is fitted with a drop dispenser tip. (To remove the drop dispenser tip, press laterally with thumb until the tip snaps off.) When dispensing drops, hold the bottle in an inverted position and squeeze gently. Secure the cap on the bottle when it is not in use.

When dispensing ImmPRESS™ reagent, apply a sufficient number of drops on the slide to cover the entire section. Slides should then be placed in a humidified chamber during the incubation period.

The staining procedure should be performed at room temperature (20 - 25 °C). The ImmPRESS™ reagent should be equilibrated to room temperature for optimal performance. After completion of the staining procedure, the reagent should be stored at 4 °C (do not freeze).

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.

### ENZYME SUBSTRATES

A variety of chromogens can be used to localize peroxidase substrates in tissue sections. Vector Laboratories offers the traditional substrates DAB and AEC as well as several proprietary substrates, producing colors as listed.

These substrates can be used as single labels or to introduce multiple colors in a tissue section.

DAB (Diaminobenzidine), SK-4100, brown  
DAB + Ni<sup>2+</sup>, SK-4100, gray/black  
Vector® VIP, SK-4600, purple  
Vector® SG, SK-4700, blue-gray  
Vector® NovaRED™, SK-4800, dark red  
TMB, SK-4400, blue  
AEC (3-amino-9-ethyl carbazole)\*, SK-4200, red

Vector Laboratories also offers a line of peroxidase substrates with increased sensitivity.

ImmPACT™ DAB, SK-4105, brown  
ImmPACT™ VIP, SK-4605, purple  
ImmPACT™ SG, SK-4705, blue-gray  
ImmPACT™ NovaRED™, SK-4805, dark red  
ImmPACT™ AEC\*, SK-4205, red

\* AEC is soluble in alcohol and clearing agents and must be mounted in aqueous mounting media. All other substrates are not soluble in alcohol or clearing agents. They may be dehydrated, cleared, and permanently mounted.

### STAINING PROCEDURE FOR PARAFFIN SECTIONS

1. Deparaffinize and hydrate tissue sections through xylenes or other clearing agents and graded alcohol series.
2. Rinse for 5 minutes in tap water.\*
3. If quenching of endogenous peroxidase activity is required, incubate the sections for 30 minutes in 0.3% H<sub>2</sub>O<sub>2</sub> in either methanol or water. Incubation times may be shortened by using higher concentrations of H<sub>2</sub>O<sub>2</sub>. If endogenous peroxidase activity does not present a problem, this step may be omitted.
4. Wash in buffer for 5 minutes.
5. When necessary, incubate sections for 20 minutes with ready-to-use (2.5%) normal horse blocking serum or blocking solution of choice.
6. Incubate sections with rabbit primary antibody diluted in appropriate antibody diluent solution. (See Note 5)
7. Wash slides for 5 minutes in buffer.
8. Incubate sections for 30 minutes with ImmPRESS™ reagent.
9. Wash slides for 5 minutes in buffer.
10. Incubate sections in peroxidase substrate solution until desired stain intensity develops. (See Note 3)
11. Rinse sections in tap water.

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12. Counterstain, clear and mount.

\* If antigen unmasking is required, perform this procedure after step 2, using Cat. No. H-3300 (citrate-based) or H-3301 (high pH).

### STAINING PROCEDURE FOR FROZEN SECTIONS

This procedure is generally appropriate for frozen sections, cell smears or cytocentrifuge preparations.

1. Sections are air dried.
2. Immediately before staining, fix sections with acetone or the appropriate fixative for the antigen under study.
3. Transfer slides into buffer.
4. If quenching of endogenous peroxidase is required, use gentle H<sub>2</sub>O<sub>2</sub> blocking to reduce the risk of antigen destruction or tissue loss: 0.3% H<sub>2</sub>O<sub>2</sub> in 0.3% normal serum in PBS for 5 minutes; or 0.3% H<sub>2</sub>O<sub>2</sub> in methanol for 30 minutes, or use other published methods (eg. Andrew, S. M., Jasani, B., *Histochem J.* 1987, **19**, 426-30).
5. Follow steps 4-12 of the procedure recommended for paraffin sections.

For best results, **ImmPRESS™** reagent should be used before the expiration date.

#### NOTES:

1. **ImmPRESS™** reagents can be used in multiple antigen labeling applications. A brochure is available with protocols - "Discovery through color". Please request a printed copy or download from our website: [www.vectorlabs.com](http://www.vectorlabs.com).
2. Solutions containing sodium azide or other inhibitors of peroxidase activity should not be used in diluting the peroxidase substrate.
3. Substrate development times may differ depending upon the level of antigen, the intensity of the stain that is required, or the substrate used. **ImmPACT™** DAB and **DAB** generally should be developed for 2-10 minutes; **ImmPACT™** VIP and **Vector®** VIP for 2-15 minutes; **ImmPACT™** SG and **Vector®** SG for 2-10 minutes; **ImmPACT™** NovaRED™ and **Vector®** NovaRED™ for 2-15 minutes; **ImmPACT™** AEC and **AEC** for 10-30 minutes; **TMB** for 5-20 minutes. Some counterstains may not be compatible with certain peroxidase substrates because of solubility of the reaction products or lack of color contrast. A counterstain compatibility chart is available upon request. Refer to the instructions in the respective substrate kits for further details.

4. The section should be well prepared. Fixation (generally, in buffered formalin not exceeding 4 percent formaldehyde) should be sufficient to maintain the integrity of the section throughout the staining procedure but not so harsh as to destroy the antigen under study. During the staining procedure, do not allow the section to dry out. Use a humidified chamber for incubations. In some cases, use of an Antigen Unmasking Solution, Cat. No. H-3300 (citrate-based) or Cat. No. H-3301 (high pH) and exposure to high temperatures can overcome loss of antigens due to fixation.
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 or 0.1% immunohistochemical grade bovine serum albumin (Cat. No. SP-5050). Only immunohistochemical grade BSA should be used, as other preparations can contain undesired impurities.
6. 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 the substrate solution be prepared with glass distilled water. Deionized water (even with low conductivities) may contain inhibitors of peroxidase and can reduce sensitivity.
7. Sections of neuronal tissue or sections which are thicker than normal may require longer incubation times for optimal staining.
8. 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.
9. After mounting, paraffin sections should be dried in a hot air oven at 50-56 °C. Some slide warmers contain "hot spots" that can overheat tissues.
10. Specimens should not be embedded in paraffin heated higher than 60 °C. Too much heat can destroy antigens.
11. Hand lotions can cause sections to detach from slides or may prevent adequate penetration of reagents. Avoid touching rinse baths with oily hands.
12. Paraffin tissue blocks should be stored in sealed containers in a cool location.
13. Complete deparaffinization is important. Clearing agents and alcohol solutions should be changed regularly. All steps of the deparaffinization should be sufficiently long to completely remove the paraffin from the sections.
14. To reduce the risk of introducing contaminants, avoid removing the drop dispenser from the stock solution bottle unless dispensing large volumes. Avoid pipetting **ImmPRESS™** reagent directly from the bottle.

### Peroxidase Substrates

These kits provide sufficient stock reagents to prepare about 300 ml of substrate solution:

<b>DAB Substrate</b>	1 Kit	<b>SK-4100</b>
<b>Vector® VIP Substrate</b>	1 Kit	<b>SK-4600</b>
<b>Vector® SG Substrate</b>	1 Kit	<b>SK-4700</b>
<b>Vector® NovaRED™ Substrate</b>	1 Kit	<b>SK-4800</b>
<b>TMB Substrate</b>	1 Kit	<b>SK-4400</b>
<b>AEC Substrate</b>	1 Kit	<b>SK-4200</b>

More sensitive versions of most of these substrates are also available as **ImmPACT™** substrates:

<b>ImmPACT™ DAB</b>	120 ml	<b>SK-4105</b>
<b>ImmPACT™ VIP Substrate</b>	120 ml	<b>SK-4605</b>
<b>ImmPACT™ SG Substrate</b>	120 ml	<b>SK-4705</b>
<b>ImmPACT™ NovaRED™ Substrate</b>	120 ml	<b>SK-4805</b>
<b>ImmPACT™ AEC Substrate</b>	120 ml	<b>SK-4205</b>

Other related reagents also available are:

<b>ImmPRESS™ Reagent Anti-Mouse Ig</b>	Kit	<b>MP-7402</b>
<b>ImmPRESS™ Reagent Anti-Mouse Ig (rat adsorbed)</b>	Kit	<b>MP-7422</b>
<b>ImmPRESS™ Reagent Anti-Rat Ig</b>	Kit	<b>MP-7404</b>
<b>ImmPRESS™ Reagent Anti-Rat Ig (mouse adsorbed)</b>	Kit	<b>MP-7444</b>
<b>ImmPRESS™ Reagent Anti-Goat Ig</b>	Kit	<b>MP-7405</b>
<b>ImmPRESS™ Universal Reagent Anti-Mouse/Rabbit Ig</b>	Kit	<b>MP-7500</b>
<b>VectaMount™ Mounting Medium</b>	60 ml	<b>H-5000</b>
<b>VectaMount™ AQ Mounting Medium</b>	60 ml	<b>H-5501</b>
<b>Antigen Unmasking Solution (100x) Citrate-based</b>	250 ml	<b>H-3300</b>
<b>High pH</b>	250 ml	<b>H-3301</b>
<b>Bovine Serum Albumin (BSA)</b>	500 mg	<b>SP-5050</b>
<b>VECTABOND™ Reagent</b>	7 ml	<b>SP-1800</b>
<b>Vector® Hematoxylin</b>	500 ml	<b>H-3401</b>
<b>Vector® Hematoxylin QS</b>	100 ml	<b>H-3404</b>
<b>Vector® Methyl Green</b>	500 ml	<b>H-3402</b>
<b>Vector® Nuclear Fast Red</b>	500 ml	<b>H-3403</b>
<b>ImmEdge™ Hydrophobic Barrier Pen</b>	2-pen set	<b>H-4000</b>
<b>ImmPrint™ Histology Pen</b>	5-pen set	<b>H-6100</b>
<b>Rabbit IgG Control Antibody</b>	5 mg	<b>I-1000</b>

A complete catalog listing is available upon request.

Visit our website: [www.vectorlabs.com](http://www.vectorlabs.com)

