



ImmPRESS™ REAGENT Anti-Rat Ig

PEROXIDASE
Cat. No. MP-7404

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 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-rat Ig reagent contains a “micropolymer” of a very active peroxidase coupled to our affinity-purified anti-rat IgG (H+L) secondary antibody. The ImmPRESS™ reagent is supplied prediluted in a convenient dropper bottle. No mixing or titering of ImmPRESS™ reagents is necessary to obtain optimal immunohistochemical staining. Ready-to-use 2.5% normal goat 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 + Ni²⁺, 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% H₂O₂ in either methanol or water. Incubation times may be shortened by using higher concentrations of H₂O₂. 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 goat blocking serum or blocking solution of choice.
6. Incubate sections with rat 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 H₂O₂ blocking to reduce the risk of antigen destruction or tissue loss: 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).
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 free printed copy or download from our website: 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 goat 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:

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

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

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

Other related reagents also available are:

ImmPRESS™ Reagent Anti-Rabbit Ig	Kit	MP-7401
ImmPRESS™ Reagent Anti-Mouse Ig	Kit	MP-7402
ImmPRESS™ Reagent Anti-Mouse Ig (rat adsorbed)	Kit	MP-7422
ImmPRESS™ Reagent Anti-Rat Ig (mouse adsorbed)	Kit	MP-7444
ImmPRESS™ Reagent Anti-Goat Ig	Kit	MP-7405
ImmPRESS™ Universal Reagent Anti-Mouse/Rabbit Ig	Kit	MP-7500
VectaMount™ Mounting Medium	60 ml	H-5000
VectaMount™ AQ Mounting Medium	60 ml	H-5501
Antigen Unmasking Solution (100x) Citrate-based	250 ml	H-3300
High pH	250 ml	H-3301
Bovine Serum Albumin (BSA)	500 mg	SP-5050
VECTABOND™ Reagent	7 ml	SP-1800
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
ImmEdge™ Hydrophobic Barrier Pen	2-pen set	H-4000
ImmPrint™ Histology Pen	5-pen set	H-6100
Rat IgG Control Antibody	1 mg	I-4000

A complete catalog listing is available upon request.

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