3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Immunohistochemistry Procedure

Preparation Instructions
Citrate Buffer – 10 mM sodium citrate buffer, pH 6.0

1× Tris/EDTA Solution – 10 mM Tris base with 1 mM EDTA solution and 0.05% TWEEN® 20, pH 9.0

2 M HCl Solution

Procedure
This immunohistochemical procedure is for deparaffinized sections, rehydrated with PBS.

Pretreat the sample using **one** of the following options:

- No treatment at all
- Place sample in Citrate Buffer, pH 6.0, microwave at 750 W for 20 minutes, and then cool the sample.
- Place sample in 1× Tris/EDTA Solution, microwave at 750 W for 20 minutes, and then cool the sample.
- Place sample in 2 M HCl Solution at room temperature for 10–20 minutes.
- Place sample in 0.1% trypsin and shake for 25 minutes at 37 °C.

Immunohistochemical Procedure

1. Incubate deparaffinized, pretreated sections in 3% (v/v) H_2O_2 in 1× PBS at room temperature for 10 minutes and then wash the sections again.
2. Incubate sections in blocking solution for 10 minutes.
3. Add primary antibody diluted in blocking solution and incubate the sections overnight at 2–8 °C, then wash sample with 1× PBS.
4. Incubate sections with peroxidase labeled polymer conjugated to a secondary antibody for 30 minutes followed by washing the sections with PBS.
5. Application of substrate solution (DAB or other suitable peroxidase substrate). Wash sample thoroughly under running tap water.
6. Counterstain the samples in Mayer's hematoxylin.
7. Dehydrate and mount samples.

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