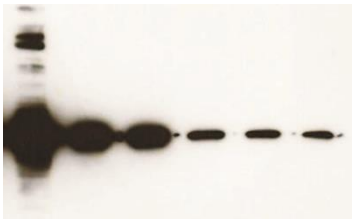
Technical Info

Well advised with Roth.



Immunostaining by HRP and AP

Protocols

Staining by Peroxidase (in Solution)

Detection of peroxidase activity (Immunoassay):

Dissolve 1 mg TMB (Art. No. 6350) in 0.1 ml dimethylsulfoxide (Art. No. 4720).

Add 9.9 ml of a 0.1 M sodium acetate solution (pH 6.0) (Powder: Art. No. 6779), filter and add H_2O_2 (Art. No. 8070) (final concentration 0.01 %).

Always prepare freshly!

Incubate 10-30 mins at room temperature (approx. 50 µl per microtitre well) finally add 50 µl 1 M H_2SO_4, Art. No. X873, per well

Photometric quantitation at 450 nm.

Reference: Bos, E.S. *et al.,* (1981) *J. Immunoassay* 2:187.

Staining by Alkalic Phosphatase (Precipitate)

Assay protocol for use of BCIP/NBT in immunoblot procedures:

Stock solutions (all solutions are stable for at least 1 year at 4 °C):

0.5 g NBT (Art. No. 4421) in 10 ml 70 % dimethylformamide (Art. No. T921);

0.5 g BCIP p-toluidine salt (Art. No. 6368) in 10 ml 100 % dimethylformamide.

Incubation buffer for alkaline phosphatase:

100 mM NaCl, 5 mM $MgCl_2$,100 mM Tris (pH 9.5).

Fresh substrate solution:

66 µl NBT stock solution + 10 ml incubation buffer, mix well,

add 33 µl BCIP stock solution. Use within 1 hour.

Blot development:

Approx. 10 ml substrate solution per 15 x 15 cm^2 membrane surface.

Develop at room temperature until bands become visible (approx. 30 mins).

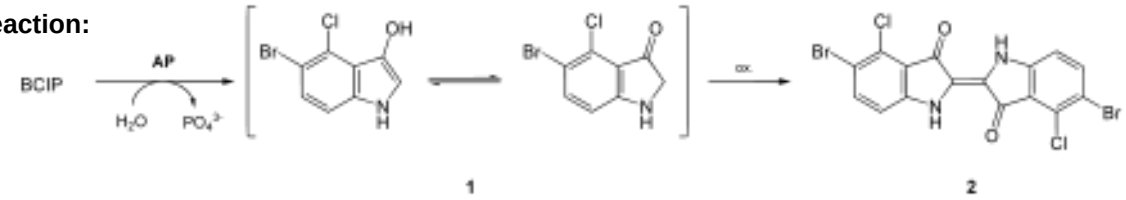
Reaction stop:

Rinse with PBS/20 mM EDTA.

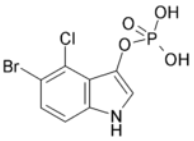
NBT / BCIP Reaction Mechanisms

Alkalic phosphatase mediated reaction of 5-bromo-4-chloro-3-indolylphosphate (BCIP or X-Phosphate) and Nitroblue tetrazolium salt (NBT). By enzymatic dephosphorylation, BCIP is converted to indoxyl which subsequently tautomerises to the ketone. In basic pH values, this ketone dimerises, resulting in a weak blue colour. During dimerisation, the ketone also releases H^+ ions, which reduce the colour enhancer NBT to purple-coloured diformazan. Both dyes precipitate in the direct vicinity of the phosphatase, hence staining this site in a dark violet.

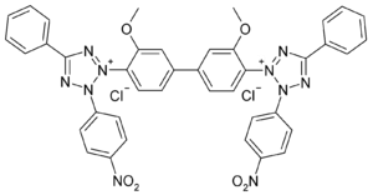
Reaction:



BCIP:



NBT:



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