Product specification

Streptavidin-alkaline phosphatase
RPN 1234

Safety warnings and precautions

Warning: For research use only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

We recommend that this product and components are handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. As all chemicals should be considered as potentially hazardous, it is advisable when handling chemical reagents to wear suitable protective clothing, such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In case of contact with skin or eyes, wash immediately with water.
Warning: Contains sodium azide in dilute solution.
Dispose of waste by flushing with copious amounts of water to avoid the building up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 1mg.

Streptavidin-alkaline phosphatase is formed by the conjugation of alkaline phosphatase and streptavidin. It is suitable for the detection of biotinylated antibodies and other biotinylated molecules in immunocytochemical, immunoblotting and ELISA techniques.

Preparation

Streptavidin-alkaline phosphatase is prepared using a single-step glutaraldehyde coupling reaction to link calf intestinal alkaline phosphatase to streptavidin. Excess coupling reagent is removed using extensive dialysis. The final product concentration is optimized to a standard titre as determined by its binding to biotinylated protein. As a result the final protein concentration may vary minimally between batches.

Quality control

In the quality control test the conjugate is tested at a range of concentrations in an ELISA test.

Plastic microtitration plates are coated with 50ng/well of biotinylated protein in 0.1M carbonate/bicarbonate buffer, pH9.5. Non-specific binding sites are blocked with 0.25%(w/v) gelatin in Tris buffered saline (TBS) pH7.6.

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The plates are then washed with TBS containing 1%(w/v) Tween^{TM}20. Conjugate is diluted across the plate to 22.5ng/ml in TBS containing 0.25%(w/v) gelatin and the plate incubated at 37°C for 1 hour. The plate is then washed in TBS containing 0.1%(w/v) Tween 20. Substrate solution (p-nitrophenol phosphate in 10mM diethanolamine buffer pH9.5, 5mM magnesium chloride, 100µl) is added to each well and the plate incubated at 37°C for 1 hour. After this time the reaction is stopped with 50µl/well of 10mM ethylenediamine tetraacetic acid (EDTA), and the absorbance at 405nm determined.

Formulation

The conjugate is supplied in 2ml of a solution containing 10mg/ml BSA, 50mM Tris-HCl, 2mM $MgCl_2$ and 0.05% sodium azide at pH8.0.

Applications

Streptavidin-alkaline phosphatase can be used to detect biotinylated molecules such as antibodies, in a variety of applications, for example, immunocytochemistry, ELISA and immunoblotting.

Immunocytochemistry:
A working dilution of 1:150 in TBS is sufficient for most applications. At this dilution approximately 3000 tests (100µl per slide) can be performed from one pack of RPN 1234. It is recommended that TBS be used in all dilutions and washings, rather than phosphate buffered saline (PBS), in order to maintain maximal enzyme activity.

ELISA:
A working dilution of 1:5000 in TBS is sufficient to detect biotinylated protein coated at 16ng/well on a microtitration plate. At this dilution, approximately 100000 assays (100µl per well) can be performed from one pack of RPN 1234.

In the QC laboratories it has been found that a 1:1000 dilution of RPN1234 will detect less than 10ng of biotinylated protein using the assay described above.

It is important to note that the activity of alkaline phosphatase depends markedly on the substrate buffer used. Studies in our laboratories have shown that, using diethanolamine/HCl pH9.5, the activity is approximately twice that obtained when using glycine/HCl pH9.5. It is therefore recommended that 3mM p-nitrophenol phosphate in 0.1M diethanolamine/HCl pH9.5, 5M magnesium chloride be used.

Immunoblotting:

A working dilution of 1:3000 (in TBS) is sufficient for most applications. At this dilution approximately 1200 tests (5ml per strip 1x10cm) can be performed from one pack of RPN 1234.

Storage and stability

Store at 2-8°C. Under these conditions the product is stable for at least 6 months.

Substrate systems

Immunocytochemistry:

New fuchsin method[4] - Prepare the reagent just before use.

1) 20ml, Tris/HCl pH9.0 (0.2M), containing magnesium chloride (5mM)
2) 5mg naphthol-AS-BI-phosphate
3) 100µl dimethylformamide
4) 250µl sodium nitrite (0.6M) - must be freshly made
5) 250µl new fuchsin (4%(w/v) in 2M HCl). This can be stored at 2-8°C.

Dissolve 2 in 3, mix 4 and 5, then add both solutions to 1, to form a yellow coloured solution, and mix well. Apply to the section and incubate for 5-30 minutes at room temperature.

Slides may be counterstained, dehydrated, cleared and mounted in a permanent mounting media.

Note: If cryostat sections are being immunostained, it may be necessary to inhibit endogenous alkaline phosphatase by adding 1mM levamisole to the final incubating medium. This will inhibit all alkaline phosphatase except the intestinal enzyme. Addition of levamisole does not alter the pH of the incubating medium.

ELISA:

Nitrophenol phosphate methods[5]- prepare the reagent just before use.

Dissolve 0.1mM p-nitrophenol phosphate in 0.1M diethanolamine/HCl pH9.5, containing 5mM magnesium chloride. Incubate for 1 hour at room temperature. The reaction may be stopped with 0.1M ethylene diamine tetra acetic acid (EDTA). The yellow reaction product absorbs maximally at 405nm.

Immunoblotting:

NBT/BCIP method[6]- prepare the reagent just before use.

1) 10ml 0.1M diethanolamine/HCl pH9.5 containing 5mM magnesium chloride.
2) 3.3mg Nitro blue tetrazolium (NBT) dissolved in 44µl of 70%(w/v) dimethylformamide.

3) 1.65mg bromo-chloro-indolyl phosphate (BCIP) in 33µl of 100%(w/v) dimethylformamide.

Add both 2 and 3 to solution 1, mix well and use immediately.
Incubate at room temperature until a reaction product is seen.

References

1) MOSSNER, E. *et al., Hoppe-Sylers Z Physiol. Chem.*, **361**, p.543, 1981.
2) HAUSAMEN, T.U. *et al., Clin. Chim. Acta.*, **15**, p.241, 1967.
3) McCOMB, R.B. and BOWERS Jr., G.N., *Clin. Chem.*, **18**, p.97 1972.
4) MALIK, N.J. and DAYMON, M.E., *J. Clin. Path.*, **35**, pp.1092-1094, 1982.
5) SNYDER, S.L. *et al., Biochimie et Biophysica Acta*, **258**, pp.178-187, 1972.
6) LEARY, J.J. *et al., Proceedings of the National Academy of Science*, **80**, pp.4045-4049, 1983.

Related products

Mouse Ig, biotinylated whole antibody (from sheep)	RPN 1001
Rat Ig, biotinylated whole antibody (from sheep)	RPN 1002
Human Ig, biotinylated whole antibody (from sheep)	RPN 1003
Rabbit Ig, biotinylated whole antibody (from donkey)	RPN 1004
Streptavidin biotinylated horseradish peroxidase complex	RPN 1051
Streptavidin horseradish peroxidase conjugate	RPN 1231
Streptavidin fluorescein	RPN 1232
Streptavidin Texas Red™	RPN 1233
^{125}I Streptavidin	IM 236
Mouse IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 931
Rabbit IgG, horseradish peroxidase linked whole antibody (from donkey)	NA 934
Rat IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 932
Human IgG, horseradish peroxidase linked whole antibody (from sheep)	NA 933
Mouse IgG, horseradish peroxidase linked F(Ab′)$_2$ fragment (from sheep)	NA 9310
Rabbit IgG, horseradish peroxidase linked F(Ab′)$_2$ fragment (from donkey)	NA 9340
Rat IgG, horseradish peroxidase linked F(Ab′)$_2$ fragment (from sheep)	NA 9320

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