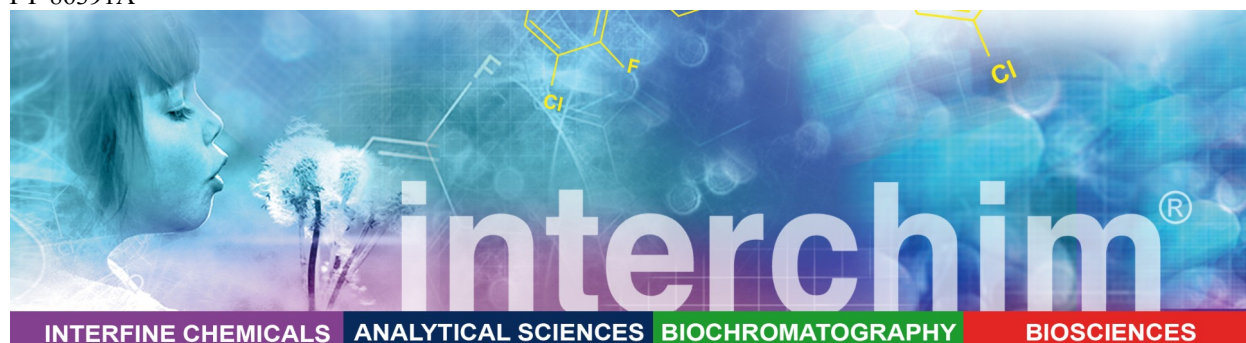


FT-86391A

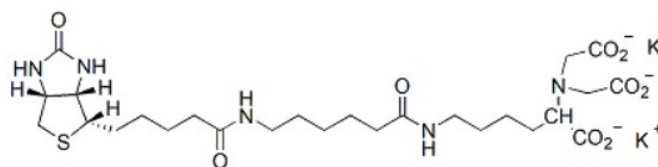


Biotin-X-NTA

Probe to detect histidine-tagged proteins immobilized on membranes

Product Description

Name :	Biotin-X NTA Biotin-X nitrilotriacetic acid, tripotassium salt
Catalog Number :	FP-86391A, 5 mg
Molecular Formula:	C ₂₆ H ₄₀ K ₃ N ₅ O ₉ S CAS 856661-92-2
MW :	715.98
Solubility:	Soluble in Water
Storage :	+4°C. Protect from moisture



Directions for use

Biotin-X NTA is used to detect histidine-tagged proteins (for as little as less than 0.1 pmol) immobilized on nitrocellulose membranes. Biotin-X NTA can be removed from the histidine-tagged protein at pH 4.8, allowing the blot to be reanalyzed with another probe.

Protocol for detection of His-tagged Protein in PVDF

- 1) Prepare your PVDF blots as needed (block nonspecific binding sites and wash the blot).
- 2) Dissolve the Biotin-X NTA in DMSO or H₂O at concentration of 1 mg/mL
- 3) Prepare fresh Staining Solution (less than 30 minutes before use): prepare 20 mL of Staining Solution for each 8 cm × 10 cm blot by adding 20 µL of 10 mM NiCl₂, 20 µL of 1 mg/mL biotin-X NTA (from Step 2) and 1-2 µL of 1 mg/mL streptavidin-alkaline phosphatase (made in step 1.2) to 20 mL of Blocking Buffer. Mix well. Once the solution is made, it is ready to use right away, and remains stable for at least 30 minutes.
- 4) Incubate the blot with 20 mL of Staining Solution (from Step 3) at room temperature for 30 minutes.
- 5) Wash the blots, and pursue for chemiluminescence detection.

Protocol for Biotin-X NTA–Probed Blot Stripping

- 1) If desired, the blot can be stained using other detection methods.
- 2) For staining with antibodies or lectins, strip the biotin-X NTA complex off the blot by incubating it in 62.5 mM Tris, 0.2% SDS, 50 mM dithiothreitol (DTT), pH 6.8, at 50°C for 40 minutes with gentle agitation. Or incubated overnight in 200 mM acetate acid with 40 mM EDTA, pH 4.8.
- 3) Wash the blot in Wash Buffer at room temperature 2-4 times for 5 minutes each and proceed with antibody detection.

Other protocol may be found in the litterature.

Bibliographic References

- **McMahan S. *et al.***, Single-step synthesis and characterization of biotinylated nitrilotriacetic acid, a unique reagent for the detection of histidine-tagged proteins immobilized on nitrocellulose, *Anal. Biochem.* 236:101 (1996)
- **O'Shannessy D. *et al.***, Detection and quantitation of hexa-histidine-tagged recombinant proteins on western blots and by a surface plasmon resonance biosensor technique, *Anal. Biochem.* 229:119 (1995)

Related items

- Alkaline Phosphatase Streptavidin, FP-518498

Ordering information

Catalog size quantities and prices may be found at <http://www.interchim.com>.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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