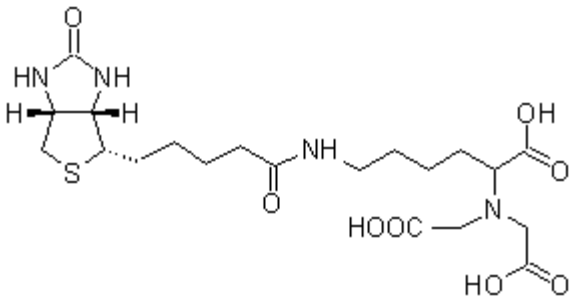
Biotin-X nitrilotriacetic acid, potassium salt

Ordering Information	*Storage Conditions*
Product Number: 3009 (1 mg)	Keep at -20 °C and desiccated. Expiration date is 12 months from the date of receipt.

Chemical and Physical Properties



Molecular Weight: 715.98
Appearance: White powder
Solvent: dimethylsulfoxide (DMSO) or H_2O

Biological Applications

Biotin-X nitrilotriacetic acid (biotin-X NTA) is widely used to detect histidine-tagged proteins immobilized on nitrocellulose membranes. The NTA moiety of biotin-X NTA chelates Ni ion that is also chelated with histidine tags. The NTA-polyHis-complex can be detected using standard enzyme-linked streptavidin methods. Biotin-X NTA can be used to detect less than 0.1 pmol of histidine-tagged protein using a streptavidin—horseradish peroxidase conjugate and chemiluminescence techniques. In combination with fluorescent avidin conjugates, this NTA biotin derivative can be used for detecting polyhistidine-containing biomolecules such as fusion proteins. This NTA Biotin derivative is a bifunctional reagent that is used to detect histidine-tagged proteins immobilized. The nitrilotriacetic acid is used to chelate a Ni(II) ion at four of its six coordination sites. The remaining two sites are available for binding to a histidine tag. The biotin functional group can then be detected using a streptavidin-horseradish peroxidase conjugate and chemiluminescence. Using this biotinylated nitrilotriacetic acid, it is possible to detect less than 0.11 pmol of histidine-tagged Escherichia coli RNA polymerase sigma70 subunit. This reagent is also able to specifically detect His-tagged sigma70 from a whole cell lysate following SDS-PAGE and transfer to nitrocellulose. The reagent can be dissociated from the His-tagged protein at pH 4.8 and the blot can be reprobed with a monoclonal antibody for detection of different proteins on the same blot.

Sample 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 H_2O 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 $NiCl_2$, 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 pursuit for chemiluminescence detection.

Sample 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.

References

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2. Bogusiewicz A, Mock NI, Mock DM. (2005) A biotin-protein bond with stability in plasma. Anal Biochem, 337, 98.
3. Huhtinen P, Soukka T, Lovgren T, Harma H. (2004) Immunoassay of total prostatespecificantigen using europium(III) nanoparticle labels and streptavidin-biotin technology. J Immunol Methods, 294, 111.
4. Addo JK, Buolamwini JK. (2004) Design, synthesis, and evaluation of 5'-S-aminoethyl-N(6)-azidobenzyl-5'-thioadenosine biotin conjugate: a bifunctional photoaffinity probe for the es nucleoside transporter. Bioconjug Chem, 15, 536.
5. McMahan, S.A. and R.R. Burgess, Single-step synthesis and characterization of biotinylated nitrilotriacetic acid, a unique reagent for the detection of histidine-tagged proteins immobilized on nitrocellulose. *Anal Biochem* 1996, **236**, 101-6.