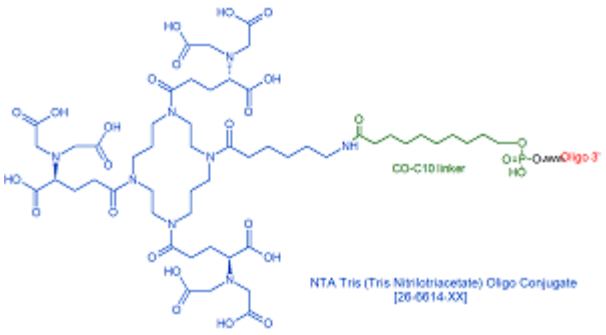
Oligo Modifications

NTA Tris

Category	Affinity Ligands
Modification Code	NTA-Tris
Reference Catalog Number	26-6614
5 Prime	Y
3 Prime	N
Internal	N
Molecular Weight(mw)	1049



Gene Link provides custom synthesis of NTA Tris (Nitrilotriacetate) Oligo Conjugate.
NTA-Tris modification is a post synthesis conjugation to an active NHS group of either a NHS-Carboxy C10 or a NHS-dT group thus an additional modification is required at the 5' end with additional charges for that modification.
Yield of Post Synthesis NHS, Maleimide & Click Ligand Conjugation* Oligo Scale of Synthesis Yield, nmols 50 nmol 2 nmol 200 nmol 5 nmol 1 umol 16 nmol 2 umol 30 nmol 5 umol 75 nmol 10 umol 150 nmol 15 umol 225 nmol * The yield will be lower for oligos longer than 50mer. Click here for yield table of long oligos. * Click here for RNA Oligos scale of synthesis and yield. **NHS Ligand conjugation** requires a primary amino group. Gene Link offers a wide selection of amino modifications for 5', 3' and internal sites.
Click here for a list of conjugation chemistry modifications. **Maleimide Ligand conjugation** requires a thiol group. Gene Link offers a wide selection of thiol modifications for 5', 3' and internal sites.
Click here for a list of conjugation chemistry modifications.

Click Chemistry Ligand conjugation requires a corresponding Click modification; examples Alkyne:Azide, Azide:DBCO, BCN:Azide,
BCN:Tetrazine and TCO:Tetrazine. Gene Link offers a wide selection of click modifications for 5', 3' and internal sites. Click here for a list of click chemistry modifications.
Nitrilotriacetic acid (NTA) is widely used in affinity chromatography to purify His-tagged proteins. The NTA chelator forms a complex with metal ions, usually Ni(2+), which then binds to histidine residues in the His-tag. Two forms of NTA are available, monovalent (NTA Mono) and trivalent (NTA Tris) differ in their binding strength and application. Tris-NTA has a significantly higher affinity for His-tags compared to mono-NTA, with affinities in the nanomolar range versus the micromolar range for mono-NTA. This higher affinity is due to the multivalency of tris-NTA, which allows it to bind the His-tag at three points, resulting in a much more stable and strong interaction. This leads to more stable baselines in experiments like SPR and improved detection sensitivity.
Comparison of NTA Mono and NTA Tris Affinity & Other Features Towards His Tag

Feature

Mono NTA

Tris NTA Structure NTA Mono: One NTA group per ligand NTA Tris: Three NTA groups per ligand (clustered) Histidine Binding Binds via 2-4 histidine residues Binds via 6+ residues, higher avidity Affinity uM range K_D nM-pM range K_D Elution Easy with imidazole/EDTA Difficult due to strong binding.
Requires 100 mM imidazole or higher Application Protein purification. Weak binding acceptable Requiring high affinity binding.
Biosensing, stable immobilization The enhanced binding of tris-NTA is a result of multivalency, where the simultaneous binding of three NTA-metal ion complexes to the histidine tag creates a much stronger overall interaction.
Applications - NTA Mono is commonly used in immobilized metal affinity chromatography (IMAC) for routine purification of His-tagged proteins. - NTA Tris provides higher binding strength and is preferred in biosensor applications such as SPR (Surface Plasmon Resonance) and BLI (Bio-Layer Interferometry).
Binding Affinity Summary

Ligand

Approximate K_D to His tag NTA Mono-Ni(2+) ~1-10 uM NTA Tris-Ni(2+) ~1-100 nM The enhanced binding of tris-NTA is a result of multivalency, where the simultaneous binding of three NTA-metal ion complexes to the histidine tag creates a much stronger overall interaction. The His-tagged protein is effectively "gripped" by three separate NTA-metal interactions. Dissociation requires breaking all three bonds simultaneously, which is statistically far less likely and requires much more energy.
References
1. 1. J. Shimada, T. Maruyama, T. Hosogi, J. Tominaga, N. Kamiya, M. Goto,. Conjugation of DNA with protein using His-tag chemistry and its application to the aptamer-based detection system, Biotechnol. Lett. 30 (2008) 2001-2006.
2.J. Shimada et al DNA enzyme conjugate that can detect thrombin. Anal. Biochem. 414 (2011) 103-108. - NTA Mono (Nitrilotriacetate) Oligo Conjugate
3. Hochuli, E., et al. (1988). Genetic Approach to Facilitate Purification of Recombinant Proteins with a Novel Metal Chelate Adsorbent. *Bio/Technology*, 6, 1321-1325
4. Lata, S., et al. (2005). High-affinity adaptors for switchable recognition of histidine-tagged proteins. *Journal of the American Chemical Society*, 127(29), 10205-10215.
5. Porath, J., et al. (1975). Metal chelate affinity chromatography, a new approach to protein fractionation. *Nature*, 258,

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