



## Product Specifications

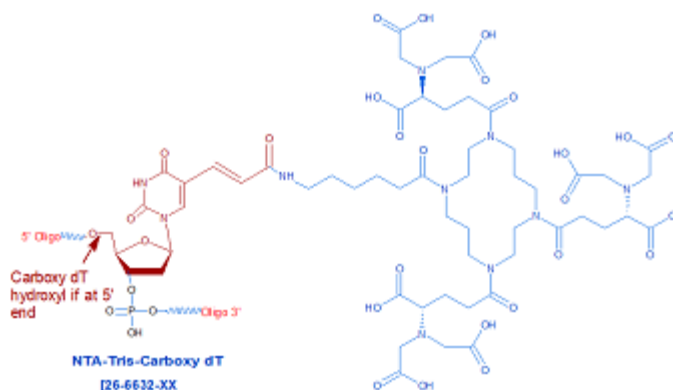
Custom Oligo Synthesis, antisense oligos, RNA oligos, chimeric oligos, Fluorescent dyes, Affinity Ligands, Spacers & Linkers, Duplex Stabilizers, Minor bases, labeled oligos, Molecular Beacons, siRNA, phosphonates Locked Nucleic Acids (LNA); 2'-5' linked Oligos

## Oligo Modifications

For research use only. Not for use in diagnostic procedures for clinical purposes.

### NTA Tris Carboxy dT Oligo

Category	Affinity Ligands
Modification Code	NTA-Tris-CO-dT
Reference Catalog Number	26-6632
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<sub>D</sub> nM-pM range K<sub>D</sub> 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<sub>D</sub> 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. 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,

598-599.