

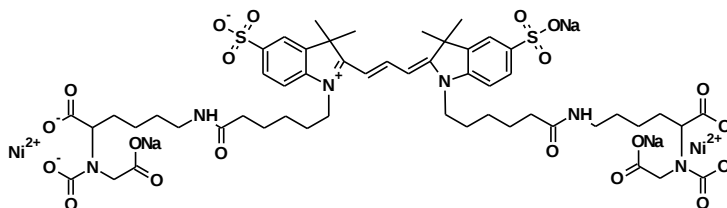
Product Information Sheet

Ordering Information

Product Number: 12613
Product Name: HIS Lite™ Cy5 Bis NTA-Ni Complex
Unit Size: 1mg
Storage Conditions: <-15 °C and kept from light and moisture
Expiration Date: 24 months upon receiving

Chemical, Physical and Spectral Properties

Molecular Weight: 1382.63
Appearance: Blue powder



Chemical Structure:
Soluble in: Water
Excitation Wavelength: 649 nm
Emission Wavelength: 665 nm

Application Notes and Protocol

Polyhistidine is one of the most popular affinity tags incorporated into recombinant proteins. It can be inserted either at the N- or C-terminus, and expressed in a variety of hosts. Due to its small size, the polyhistidine tag serves as an elegant tool for both protein purification and detection. HIS Lite™ Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes provide specific and highly sensitive detection of His-tagged fusion proteins. The Ni-NTA complexes were first reported by Kapanidis et Al. to be specific for polyhistidine tags with minimal crossreactivity. Cy3 and Cy5 dyes demonstrate strong fluorescent signals at commonly available wavelengths and with little quenching. The Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes can be directly applied either to an SDS-PAGE gel or Western blot membrane for fluorescence imaging. Detection with the Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes requires less incubation time than for protein-antibody binding. No secondary reaction is required since the Ni-NTA complexes are directly conjugated to the fluorophores.

Procedure for Direct Detection of Histidine-Tagged Proteins on SDS-PAGE Gels

Direct application of Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes to SDS-PAGE gels provides fast and easy method to monitor purified protein or protein expression in biological samples.

1. Separate His-tagged protein on a SDS-PAGE gel
2. Optional: Fix the SDS-PAGE gel in 40% ethanol and 10% acetic acid overnight at room temperature (RT)
3. Wash the gel 3 times in water
4. Incubate the gel in the dark for 1 hr with Cy3 Bis NTA-Ni or Cy5 Bis NTA-Ni Complexes in water at 1 µg/mL-0.1 µg/mL
Cy3 Bis NTA-Ni or Cy5 Bis NTA-Ni Complexes can be diluted in water to make 1 mg/mL stock.
5. Wash the gel 3 times in water
6. Image the gel at 540 nm/590 nm for Cy3 Bis NTA-Ni Complex labeling and at 640 nm/680 nm for Cy5 Bis NTA-Ni Complex labeling

Procedure for Detection of Histidine-Tagged Proteins after Western Blot Transfer

After separating proteins on SDS-PAGE gels, proteins can be transferred to low-fluorescence PVDF membranes. Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes offer a highly specific detection with less procedures than antibody based detection. Application of Cy3 Bis NTA-Ni and Cy5 Bis NTA-Ni Complexes to the membrane yields sensitivity comparable to direct gel detection.

1. Separate His-tagged proteins on a SDS-PAGE gel
2. Transfer the proteins from the SDS-PAGE gel to a low fluorescence PVDF membrane using standard transfer protocols
3. Block the membrane with 5% BSA in PBS overnight at 4 °C
4. Wash the membrane 3 times using PBS-T (1X PBS with 0.05% Tween-20)
5. Incubate the membrane in the dark for 1 hr with Cy3 Bis NTA-Ni or Cy5 Bis NTA-Ni Complexes in PBS-T at 1 µg/mL-0.1 µg/mL
Cy3 Bis NTA-Ni or Cy5 Bis NTA-Ni Complexes can be diluted in water to make 1 mg/mL stock.
6. Wash the membrane 3 times in PBS-T
7. Image the membrane at 540 nm/590 nm for Cy3 Bis NTA-Ni Complex labeling and at 640 nm/680nm for Cy5 Bis NTA-Ni Complex labeling
8. Optional: The Cy3 Bis NTA-Ni Complexes can be stripped off from the membrane by incubating the gel in 20 mM EDTA for 90 minutes at RT.

References

1. Chunxia Zhao, Lance M. Hellman, Xin Zhan, Willis S. Bowman, Sidney W. Whiteheart, and Michael G. Fried. Hexahistidine-Tag-Specific Optical Probes for Analyses of Proteins and Their Interactions. *Anal Biochem.* 2010 April 15; 399(2): 237–245. doi:10.1016/j.ab.2009.12.028.