

HIS Lite™ Cy5 Bis NTA-Ni Complex

Catalog number: 12613

Unit size: 1 mg

Component	Storage	Amount (Cat No. 12613)
HIS Lite™ Cy5 Bis NTA-Ni Complex	Freeze (< -15 °C), Minimize light exposure	1 vial (1 mg)

OVERVIEW

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.

KEY PARAMETERS

Gel Imager

Emission	700/50 nm
Excitation	Red laser

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles

HIS Lite™ Cy5 Bis NTA-Ni Complex Stock Solution

1. Prepare a 5 to 10 mM stock solution by adding an appropriate amount of DMSO.

Note: Store any unused stock solution at -20 °C. Avoid repeated freeze-thaw cycles and minimize light exposure.

PREPARATION OF WORKING SOLUTION

HIS Lite™ Cy5 Bis NTA-Ni Complex Working Solution

1. Prepare a 1 to 10 μM HIS Lite™ Cy5 Bis NTA-Ni Complex working solution in PBS.

Note: Ensure that there is sufficient working solution to fully submerge the gel. After use, discard the working solution. Do not reuse.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol should be used only as a guideline and may require optimization to better suit your specific experimental needs.

Post-run Gel Staining Protocol

1. Run gels based on your standard protocol.
2. Place the gel in a suitable container. Fix the gel in the fixing solution for 60 minutes. Note: 40% ethanol + 10% acetic acid can be used as a fixing solution.
3. Wash the gel twice with the ultra-pure water.
4. Incubate the gel in the HIS Lite™ Cy5 Bis NTA-Ni Complex working solution for 60 minutes.

Note: Be sure to fully submerge the gel in the working solution.
5. Remove the working solution and wash the gel twice with PBS.
6. Proceed to imaging the gel immediately.

For In Vitro Complex Formation

1. Mix the His-tagged protein solution and the HIS Lite™ Cy5 Bis NTA-Ni Complex working solution at the appropriate concentrations.

Note: Optimization of the HIS Lite™ Cy5 Bis NTA-Ni Complex to the His-tagged protein mix must be performed for better labeling.

Note: 1 to 10 μM of HIS Lite™ Cy5 Bis NTA-Ni Complex can be used as a starting concentration.

Note: The reaction can be performed in a buffer containing 50 mM HEPES/KOH, pH 7.4, 100 mM KCl, 1 mM MgCl₂, 2 mM β-mercaptoethanol, 5% glycerol, or a buffer of your choice.

2. Mix can be incubated for 30 minutes at room temperature or 4 °C.

Note: Optimization of the incubation time and conditions must be performed for better labeling

3. Mix can then be subjected to column purification or any other downstream process.

EXAMPLE DATA ANALYSIS AND FIGURES

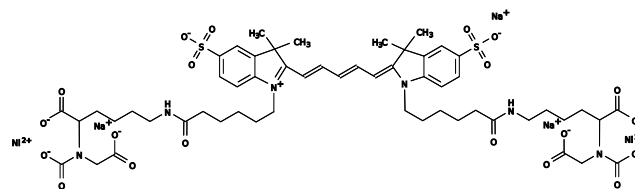


Figure 1. Chemical structure for HIS Lite™ Cy5 Bis NTA-Ni Complex

DISCLAIMER

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