HIS Lite™ iFluor® 647 Tris NTA-Ni Complex

Catalog number: 12618
Unit size: 100 ug

Component	Storage	Amount (Cat No. 12618)
HIS Lite™ iFluor® 647 Tris NTA-Ni Complex	Freeze (< -15 °C), Minimize light exposure	1 Vial (100 ug)

OVERVIEW

iFluor 647-Tris NTA compound is used as a sensitive fluorescent probe for detecting polyhistidine-labeled proteins in cells, solution and solid surfaces. It has much stronger fluorescence than the other similar wavelength of NTA compounds. In combination with other color tris-NTA compounds (such as #12615 and #12617), it can be used for multicolor analysis of polyhistidine-tagged proteins. Fluorescent tris-NTA compounds provide an efficient method for site-specific and stable noncovalent fluorescence labeling of polyhistidine-tagged proteins. In contrast to the transient binding of conventional mono-NTA, the multivalent interaction of tris-NTA conjugated fluorophores form a much more stable complex with polyhistidine-tagged proteins. The high selectivity of tris-NTA compounds toward cumulated histidines enable the selective labeling of proteins in cell lysates and on the surface of live cells. Fluorescent tris-NTA conjugates can be applied for the analysis of a ternary protein complex in solution and on surfaces. The transition metal ions (e.g., Ni ion)-mediated complexation of polyhistidine-labeled proteins with fluorescent tris-NTA conjugates provides a sensitive reporter for detecting and monitoring protein-protein interactions in real time.

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™ iFluor® 647 Tris NTA-Ni Complex Stock Solution

1. Prepare a 5 to 10 mM stock solution by adding an appropriate amount of ddH2O.

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

PREPARATION OF WORKING SOLUTION

HIS Lite™ iFluor® 647 Tris NTA-Ni Complex Working Solution

1. Prepare a 1 to 10 µM HIS Lite™ iFluor® 647 Tris 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™ iFluor® 647 Tris 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™ iFluor® 647 Tris NTA-Ni Complex working solution at the appropriate concentrations.

   Note: Optimization of the HIS Lite™ iFluor® 647 Tris NTA-Ni Complex to the His-tagged protein mix must be performed for better labeling.

   Note: 1 to 10 µM of HIS Lite™ iFluor® 647 Tris 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 ℃.

   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

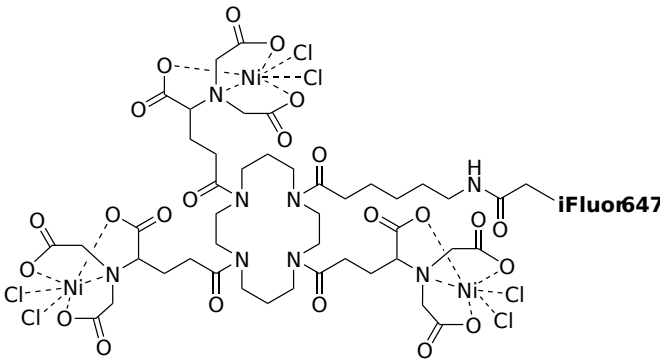

Figure 1. Fluorescent tris-NTA compounds provide an efficient method for site-specific and stable noncovalent fluorescence labeling of polyhistidine-tagged proteins. iFluor 647-Tris NTA compound is used as a sensitive fluorescent probe for detecting polyhistidine-labeled proteins in cells, solution and solid surfaces. It has much stronger fluorescence than the other similar wavelength of NTA compounds.

DISCLAIMER

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