

## HIS Lite™ iFluor® 568 Tris NTA-Ni Complex

Catalog number: 12617  
Unit size: 100 ug

Component	Storage	Amount (Cat No. 12617)
HIS Lite™ iFluor™ 568 Tris NTA-Ni Complex	Freeze (< -15 °C), Minimize light exposure	1 Vial (100 ug)

### OVERVIEW

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. This iFluor® 568 Tris NTA compound is used as a sensitive fluorescent probe for detecting polyhistidine-labeled proteins in cells, solution and solid surfaces. In combination with OG488-tris-NTA compound it can be used for multicolor analysis of polyhistidine-tagged proteins.

### KEY PARAMETERS

#### Gel Imager

Emission	602/50 nm
Excitation	Green 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® 568 Tris NTA-Ni Complex Stock Solution

1. Prepare a 5 to 10 mM stock solution by adding an appropriate amount of ddH<sub>2</sub>O.

**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® 568 Tris NTA-Ni Complex Working Solution

1. Prepare a 1 to 10 μM HIS Lite™ iFluor® 568 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® 568 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® 568 Tris NTA-Ni Complex working solution at the appropriate concentrations.

**Note:** Optimization of the HIS Lite™ iFluor® 568 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® 568 Tris NTA-Ni Complex can be used as a starting concentration.

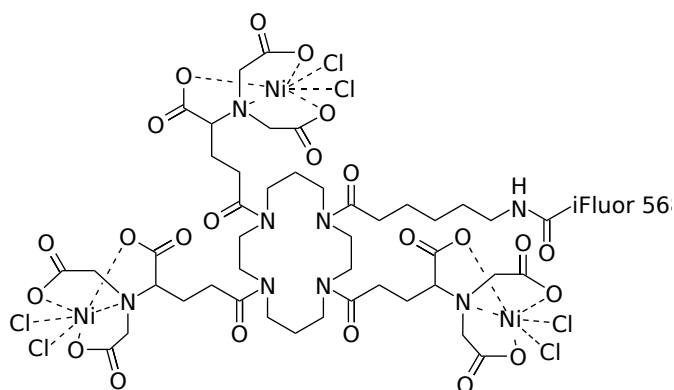
**Note:** The reaction can be performed in a buffer containing 50 mM HEPES/KOH, pH 7.4, 100 mM KCl, 1 mM MgCl<sub>2</sub>, 2 mM β-mercaptoethanol, 5% glycerol, or a buffer of your choice.

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™ iFluor® 568 Tris NTA-Ni Complex.

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