NTA-Ni-IO (ILT) binding to His tagged proteins

Introduction

His-tagged proteins can be purified and detected easily because the string of histidine residues binds to several types of immobilized metal ions (including nickel, cobalt and copper) under specific buffer conditions. In addition, anti-His-tag antibodies are commercially available for use in assay methods involving His-tagged proteins. In either case, the tag provides a means of specifically purifying or detecting the protein without the need for a protein-specific antibody or probe.

Nickel is the most widely available metal ion for purifying His-tagged proteins. For this, we developed a series of iron oxide magnetic nanocrystals with NTA-Ni on the surface (cat# ILT) that were tested to bind to His-tagged proteins and present them in a directional manner for enhanced signal-to-noise and reproducibility. This demonstrates the binding capability of NTA-Ni-IO to His-tagged proteins.

Materials:

Ocean's iron oxide magnetic nanocrystals SHP (with -COOH groups on the surface), ILP (lipid coated IO with no functional groups), ILT (-NTA-Ni groups); horse radish peroxidase (HRP) and mono-His tagged HRP as the indicator proteins; blocking buffer (cat# BBB); washing buffer (cat #WB).

Procedure:

1. **Protein binding to IO particles:** Incubate IOs diluted in washing buffer to a concentration of 1mg/mL with large molar excess HRP or His-HRP (200x for 30 nm IOs, 100x for 20 nm IOs and 50x for 10 nm IOs) at RT for 2 hr.
2. **Washing:** Wash IOs with 1 mL BBB twice and 1 mL washing buffer twice.
3. **Substrate coloration:** Determine the number of HRP on the surface of the IOs by substrate coloration. The color of substrates caused by HRP on the surface of IOs was compared with the linear range of the HRP-substrate standard curve.

Results and Discussions

The binding capacity to His tagged HPR of Ocean's iron oxide magnetic nanocrystals SHP30, ILP30 and ILT30 is presented in Table 1. The ILT30 had 20 times higher capability to distinguish between regular HRP and His tagged HRP compared with ILP30. The binding capacity to His tagged HPR of different size ILT (10 nm, 20 nm, 30 nm) is presented in Table 2.

Table 1. Binding capacity of IO magnetic nanocrystals for His tagged proteins. HRP or His-HRP was incubated with IO nanocrystals. After extensive washing steps, the number of HRP attached on the surface of the nanocrystals was determined.

Parameters	IO magnetic nanoparticles		
	SHP30	ILP30	ILT30
Number of HRP per IO particle (A)	2.61	0.70	0.01
Number of His-HRP per IO particle (B)	23.45	7.12	22.14
His-HRP binding capacity (B/A)	9.0	10.2	221.1

Table 2. Binding capacity of ILT magnetic nanocrystals for His tagged proteins. HRP or His-HRP was incubated with ILT nanocrystals. After extensive washing steps, the number of HRP attached on the surface of the nanocrystals was determined.

Parameters	IO magnetic nanoparticles		
	ILT10	ILT20	ILT30
Number of HRP per IO particle (A)	0.05	0.06	0.01
Number of His-HRP per IO particle (B)	1.03	4.22	22.14
His-HRP binding capacity (B/A)	20.57	70.33	221.14