Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B
This journal is © The Royal Society of Chemistry 2013

Electronic Supplementary Information

Ferroferric oxide /cysteine magnetic nanospheres for isolation of his-tagged proteins

Xueyan Zou,[a] Kun Li,[b] Yanbao Zhao,*[a] Yu Zhang,[b] Binjie Li[c] and Chunpeng Song[b]

ESI 1 Preparation of His-tagged proteins.

The stock solution of His-tagged protein was prepared as follows: A total of 5 mL of an overnight culture was subcultured into 300 mL fresh Luria-Bertani (LB) medium (10 g Tryptone, 5 g yeast extract and 10 g NaCl per litre of solution) containing kanamycin (50 mg/L). Transformed cells were grown at 37 °C, when the optical density (OD) at 600 reached 0.5–0.8, incubating the cells at 4 °C for 5 min. Protein expression was induced with 0.2 mM isopropyl β-d-1-thiogalactopyranoside overnight at 16 °C, 200 rpm in the rocking incubator. Cells were harvested by centrifugation at 10 °C for 15 min, 4500 rpm, resuspended in binding buffer, and disrupted by sonication. Then centrifugation at 4 °C for 40 min, 14000 rpm, the clear supernatant was filtered and the soluble His-tagged proteins were in the supernatant.

The soluble His-tagged proteins were purified as follows:

1. Preparation of Ni-NTA agarose. Use a pipette to remove sufficient slurry of Ni-NTA agarose to a 10 mL tube, sediment the medium by centrifugation for 5 min, 3000 rpm, decant the supernatant, wash the agarose by adding 5 mL Binding buffer, invert to mix, sediment the medium by centrifugation for 5 min, 3000 rpm. Repeat to add Binding buffer and sediment the medium for at least 3 times.

2. Combine the agarose with the His-tagged proteins. Add the cell lysate to the prepared Ni-NTA agarose and incubate for at least 30 min, use an agitation in the horizontal rotator. Sediment the chromatography medium by centrifugation for 5 min, 3000 rpm. Decant the supernatant, wash the Ni-NTA agarose by 5 mL Binding buffer, 5 mL Washing buffer 1, 5 mL Washing buffer 2 for at least 3 times, respectively.

3. Elute the bound His-tagged proteins. Elute the bound His-tagged proteins by adding 3 mL Elution buffer per 1 mL Ni-NTA agarose. Incubate at room temperature for 5-10 min using a horizontal rotator.

Binding buffer : 20 mM Tris, 150 mM NaCl, pH 8.0.

Washing buffer 1: 5 mM imidazole, 20 mM Tris, 150 mM NaCl, pH 8.0.

Washing buffer 2: 20 mM imidazole, 20 mM Tris, 150 mM NaCl, pH 8.0.

Elution buffer: 500 mM imidazole, 20 mM Tris, 150 mM NaCl, pH 8.0.

Water and chemicals used for buffer preparation should be of high purity. All water and solution should be filtered.

ESI 2. Concentration of His-tagged TRX washed off from different Fe_3O_4/Cys-Ni^{2+} NSs.

Fe_3O_4/Cys NSs	Thiol group density (μmol/g)	Binding Capacity (mg/g)
Sample a	189.59	53.2
Sample b	268.59	44.9
Sample c	309.62	34.4

Protein	Amount of Fe3O4/Cys (mg)	Binding Capacity (mg/g)
TRX protein	3	53.2
TRX protein	6	41.7
TRX protein	12	37.7

Amount of Fe_3O_4/Cys NSs (mg)	Concentration imidazole (mmol/L)	Binding Capacity (mg/g)
3	100	34.0
3	250	47.5
3	500	53.2
3	1000	56.5

Amount of Fe_3O_4/Cys NSs (mg)	concentration of TRX (μg/mL)	Binding Capacity (mg/g)
3	44	48.0
3	88	45.6
3	132	47.9
3	220	53.2

Amount of Fe_3O_4/Cys NSs (mg)	Reused times	Binding Capacity (mg/g)
3	1st	48.2
3	2nd	51.5
3	3rd	52.1
3	4th	53.2

Amount of Fe_3O_4/Cys NSs (mg)	His-tagged proteins	Binding Capacity (mg/g)
3	OST1	31.5
3	ABI2	28.8
3	TRX	53.2