Screening buffer conditions using His MultiTrap FF 96-well filter plates for scale-up purification of a histidine-tagged protein on ÄKTAxpress

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Buffer screening to determine optimal binding and elution conditions for purifying (histidine)$_6$-tagged NURR1 protein on His MultiTrap™ FF 96-well filter plate was performed. The buffer screening was designed to screen 96 different combinations of buffer, salt, glycerol, and reducing agents for a single protein target. Results showed that 25 mM Tris, 100 mM NaCl, 10% glycerol, pH 8.5 provided optimal binding conditions for this target protein. Using these optimal buffer conditions, purification of the target protein was scaled up using an automated, two-step purification procedure on ÄKTAxpress™. The scaled up purification revealed monomer, dimer, and trimer forms of the (His)$_6$-NURR1 target protein.

Introduction

His MultiTrap FF 96-well filter plate is designed for screening and rapid parallel purification of histidine-tagged proteins. Each well is prepacked with Ni Sepharose™ 6 Fast Flow, which is precharged with Ni^{2+} ions for convenient purification of histidine-tagged proteins. The aim of this application was to screen for optimal buffer concentrations for binding and elution of (His)$_6$-NURR1 on His MultiTrap FF 96-well filter plate. An additional aim was to scale up the purification of the target protein on ÄKTAxpress using the optimized buffer conditions determined in the buffer screening study.

Buffer screening on His MultiTrap FF

Sample buffer conditions selected for screening on His MultiTrap FF are shown in Figure 1. The sodium chloride concentration of the buffers varied from 100 to 750 mM, while the pH range chosen was 6.0 to 8.5. Glycerol (5% to 10%) and 0.05% of the reducing agent, β-mercaptoethanol, were also included in a number of sample buffers (Fig 1).

Buffer screening was performed robotically using a TECAN™ Freedom EVO™ 200 system.

E. coli expressing (His)$_6$-NURR1 was suspended in 2× concentrates of the buffers. Samples were lysed enzymatically, the lysate clarified by centrifugation, and supernatants transferred to His MultiTrap FF. Supernatants were incubated on the His MultiTrap FF plate for 10 min after which unbound protein was collected by centrifugation. His MultiTrap FF wells were washed with buffer containing 50 mM imidazole and target protein was eluted with buffer containing 500 mM imidazole.

At lower pH, weak (His)$_6$-NURR1 binding occurred, most of which was nonspecific in nature (Fig 1). At higher pH, the (His)$_6$-NURR1 binding was dependent on the NaCl concentration in the buffer. Increasing NaCl concentration decreased the amount of bound protein isolated while increasing glycerol concentration increased the amount of bound protein. The optimal buffer matrix for purification of (His)$_6$-NURR1 on His MultiTrap FF was 25 mM Tris, 100 mM NaCl, 10% glycerol, pH 8.5 (Fig 1).

96-well filter plate:	His MultiTrap FF
Sample:	*E. coli* expressing (His)$_6$-NURR1
Lysis/binding buffer:	Buffer matrix with 1× BugBuster™ Protein Extraction Reagent, 25 U/ml Benzonase™ Nuclease, 1 kU/ml rLysozyme™ Solution, and 2× Complete™ Protease Inhibitor Cocktail Tablet solution
Wash buffer:	Buffer matrix with 50 mM imidazole
Elution buffer:	Buffer matrix with 500 mM imidazole
SDS-PAGE:	4 to 12% SDS gradient

Automated, two-step purification of (His)$_6$-NURR1 using ÄKTAxpress

Using the optimized sample buffer conditions determined in the buffer screening study, a scaled up, two-step purification of the target protein on ÄKTAxpress was performed.

E. coli cells expressing (His)$_6$-NURR1 were homogenized in lysis buffer prior to purification. Lysis, binding, and elution buffers used in the purification contained 25 mM Tris, 100 mM NaCl, 10% glycerol, pH 8.5 which was the optimal condition for binding and elution. Imidazole was added to the buffer base for binding, and elution of the target protein (see Fig 2 for buffer conditions).

In the automated procedure on ÄKTAxpress, target protein was purified directly from the unclarified *E. coli* lysate first by immobilized metal affinity chromatography (IMAC) on a 1-ml HisTrap™ FF crude column, followed by final polishing using gel filtration on a HiLoad™ 16/60 Superdex™ 200 pg column. Results of the purification show that (His)$_6$-NURR1 is a mixture of monomer, dimer, and trimer under these conditions (Fig 2). SDS-PAGE of sample fractions indicate that sample purity from the ÄKTAxpress purification was high.

Columns:	IMAC, HisTrap FF crude, 1 ml Gel filtration, HiLoad 16/60 Superdex 200 pg
Sample:	Unclarified *E. coli* expressing (His)$_6$-NURR1
Lysis buffer:	25 mM Tris, 100 mM NaCl, 10% glycerol, 1 mM TCEP, 20 mM imidazole plus 25 U/ml Benzonase Nuclease, 1 kU/ml rLysozyme Solution, and 2× Complete Protease Inhibitor Cocktail Tablet solution, pH 8.5
IMAC binding and wash buffer:	25 mM Tris, 100 mM NaCl, 10% glycerol, 1 mM TCEP, 20 mM imidazole, pH 8.5
IMAC elution buffer:	25 mM Tris, 100 mM NaCl, 10% glycerol, 1 mM TCEP, 500 mM imidazole, pH 8.5
Gel filtration buffer:	25 mM Tris, 200 mM NaCl, 5% glycerol, 1 mM TCEP, pH 8.5
System:	ÄKTAxpress

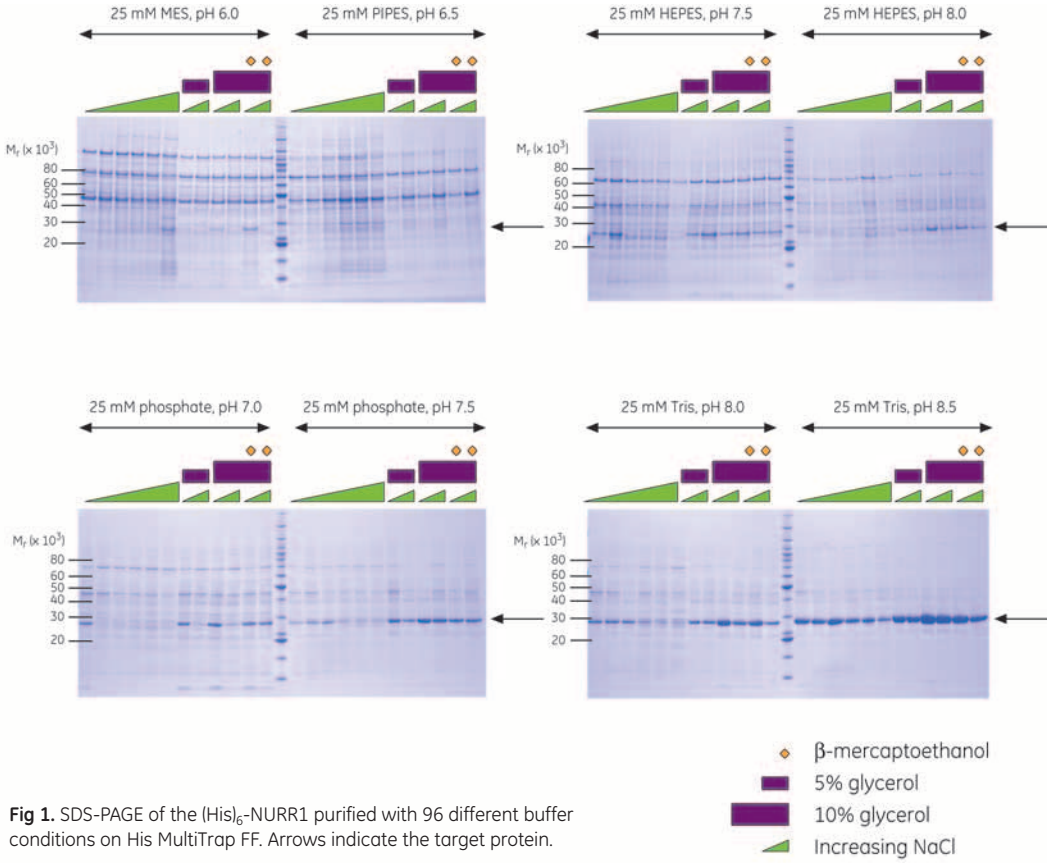

Fig 1. SDS-PAGE of the $(His)_6$-NURR1 purified with 96 different buffer conditions on His MultiTrap FF. Arrows indicate the target protein.

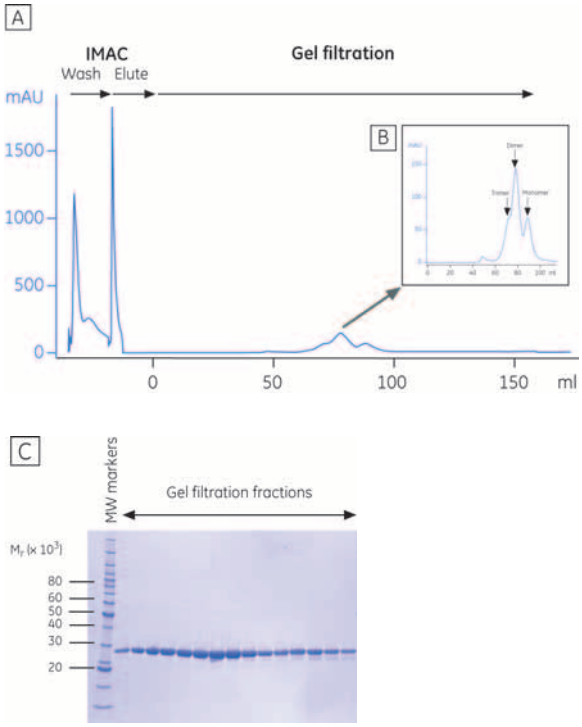

Fig 2. (A). Purification of $(His)_6$-NURR1 by automated IMAC and gel filtration on ÄKTAxpress. (B). Enlargement of the gel filtration peak shows the three isoforms of the target protein resulting from the purification. (C) SDS-PAGE of gel filtration fractions shows the high purity of the $(His)_6$-NURR1 protein obtained from the purification.

Conclusions

Optimization of buffers is easily achieved using His MultiTrap FF 96-well filter plates; the optimal buffer condition for purification of $(His)_6$-NURR1 was 25 mM Tris, 100 mM NaCl, 10% glycerol, pH 8.5. This buffer was used as the basis for making optimal buffers for larger scale purification of $(His)_6$-NURR1 on ÄKTAxpress. The scale-up, which comprised IMAC and gel filtration purification, successfully revealed three isoforms of pure $(His)_6$-NURR1 target protein.

For more information on His MultiTrap FF, visit www.gehealthcare.com/his

Ordering information

Product	Code number
His MultiTrap FF (4 × 96-well plates)	28-4009-90
Collection plate (96-well, 5 pack 500 µl, V-bottom)	28-4039-43