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Automated histidine tagged protein purification with His Mag Sepharose® Ni kit (GE® Healthcare Life Sciences) on the ep*Motion*® M5073

Introduction

Expression and purification of recombinant proteins using bacterial, mammalian, and insect expression systems are increasingly common techniques, particularly with the emerging emphasis on proteomics. Proteins expressed and purified from these systems are used in many applications, including enzymatic assays, investigation of intermolecular interactions, and structural studies.
This short protocol describes the configuration and pre-programmed method for the automation of histidine tagged protein purification on the ep*Motion* M5073 with the His Mag Sepharose Ni kit from GE Healthcare Life Sciences. His Mag Sepharose Ni products are magnetic beads designed for small scale purification/screening of his-tag proteins from different sources.

The entire procedure is being carried out in one method. The complete process is performed in 2 mL Protein Lobind tubes positioned on one epMotion PrepRack.
1 to 24 samples can be processed in parallel. The total run time for 24 samples is ~ 2 hours 40 minutes.

Sample and bead volumes used in this method are 1 mL and 200 µL, respectively. As mentioned in the instruction manual of this kit, the sample volume and bead amount, as other parameters such as sample incubation time and buffer composition, can be adapted. If the user would like to use different volumes, the method would need to be evaluated and optimized if requested.

Material and Methods

Required equipment and accessories
> ep*Motion* M5073 - TS1000 pipetting tool
> Thermorack for 24 tubes
> PrepRack for 24 Safe-Lock Tubes 2 mL
> Reservoir Rack for ep*Motion*
> Reservoir Rack Module TC, for use in ep*Motion* Reservoir Racks, temperature-controlled, 2 x reaction vessels diameter 29 mm

Required consumables and reagents
> epT.I.P.S.® Motion 1000 µL Filter
> ep*Motion* Reservoir 100 mL
> Liquid waste Tub
> Protein LoBind Tubes, 1.5 mL
> Protein LoBind Tubes, 2 mL
> 50 mL conical centrifuge tube
> His Mag Sepharose Ni kit (GE Healthcare Life Sciences; order no. 28-9799-17)
> His buffer Kit (GE Healthcare Life Sciences; order no. 28-4010-39 AC)

Method

Method Name	approx runtime (24 samples)
Application_His-tag protein purif-XXYYZZ_RRSSTT.export	2 hours 40 min

(XX=year, YY=month, ZZ=day_RR=hour, SS=minute, TT=second)

This protocol is programmed to process 1 to 24 samples in parallel. The method was developed for the ep*Motion* M5073 and can be transferred to the ep*Motion* 5075m.

Purification of histidine-tagged proteins on Immobilized-Metal Affinity Chromatography (IMAC) media is a balance between capacity and purity, modulated by the concentration of imidazole in the sample and in the binding/washing buffers. Low concentration of imidazole favors high yield, while high concentration increases purity. Depending of the goal, the user has to select the imidazole concentration used in the sample and equilibration buffer according to the kit manual. Before starting the method, the sample has to be prepared by adjusting it to the composition and pH of the equilibration buffer. This can be performed by adding buffer, NaCl and imidazole from stock solutions present in the His Buffer kit from GE Healthcare. The minimal volume per adjusted sample is 1014 µL (prepared in 1.5 mL Protein LoBind Tubes) to allow a sufficient volume to pipette 1 mL sample per tube. The sample tubes have to be positioned in a Thermorack for 24 tubes.

Required amount of equilibration buffer, beads solution and elution buffer have to be transferred respectively to epMotion reservoir of 100 mL and 2x 50 mL tubes, and positioned in a reservoir rack as described in the reservoir rack layout figure. Vortex the beads vial to homogenize the solution and immediately pipette the required amount of beads into a 50 mL tube. As the beads sediment rapidly, a resuspension of the bead solution has to be performed between each pipetting. A conical 50 mL tube is used for the bead solution as it allows a good mixing of the beads, whatever the number of samples processed are and helps for a reduction of the dead volume.

Empty protein LoBind tubes of 2 mL have to be provided in both a PrepRack and a Thermorack for 24 tubes. The number of tubes corresponds to the number of samples processed in the run.

The 2 Thermoracks, the PrepRack, the ReservoirRack, 2 boxes of epT.I.P.S. Motion 1000 µL Filter and a liquid waste tub have to be positioned on the worktable of the ep*Motion* M5073 as described in the worktable layout figure.

The method comprises the magnetic bead preparation, the bead equilibration, the sample application, the washing and the elution steps. The elution includes 2 incubation steps with 100 µL elution solution in order to increase the yield resulting in an elution fraction of ~ 195 µL.

Worktable Layout

Position	Item
TMX	PrepRack for 24 Eppendorf Safe-Lock Tubes 2 mL with empty Protein LoBind tubes of 2 mL
A2	Thermorack for 24 tubes with Protein LoBind tubes of 1.5 mL containing the sample(s)
B1	epT.I.P.S. Motion 1000 µL Filter
B2	epT.I.P.S. Motion 1000 µL Filter
C1	ReservoirRack with 1x 100 mL reservoir + 1x reservoir rack module for 2x 50 mL tubes
C2	Thermorack for 24 tubes with empty Protein LoBind tubes of 2 mL

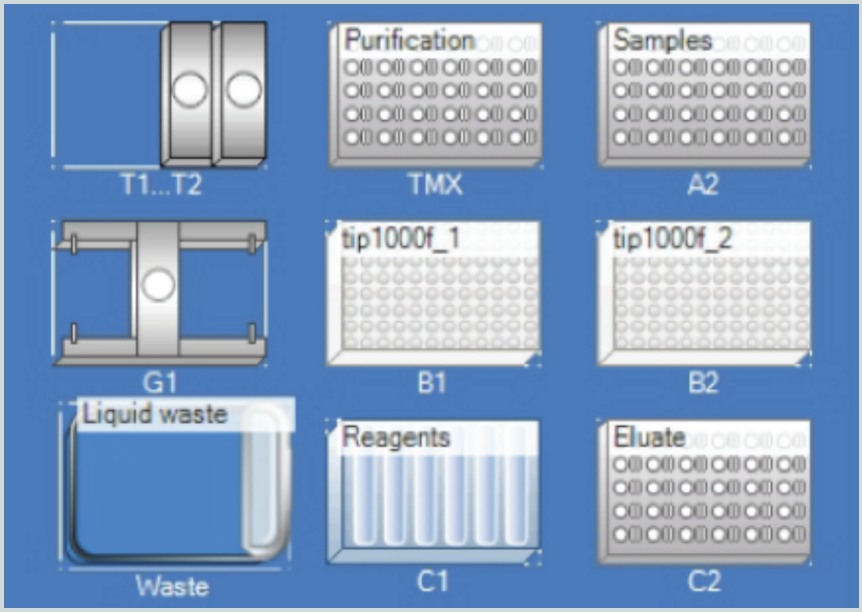

Figure 1: ep*Motion* worktable layout

Reservoir rack layout

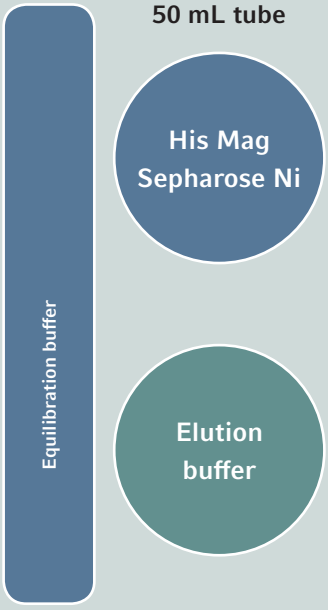

Figure 2: Reservoir rack layout

Instructions, Results and Discussion

The protein purification efficiency of this ep*Motion* method using the "His Mag Sepharose Ni" was evaluated on E. *coli* lysate spiked with 100 µg histidine-tagged GFP proteins. An imidazole concentration of 40 mM was selected as it was shown by GE healthcare that the best compromise between his-tag GFP-(His)6 extraction yield and purity is obtained with 40 mM imidazole. Side-by-side comparison of manual and automated process is presented on figure 1. Based on the SDS-PAGE gel analysis of the eluate fractions, it was demonstrated that the automated process on the ep*Motion* gives similar yield and purity as the manual process (figure 1).

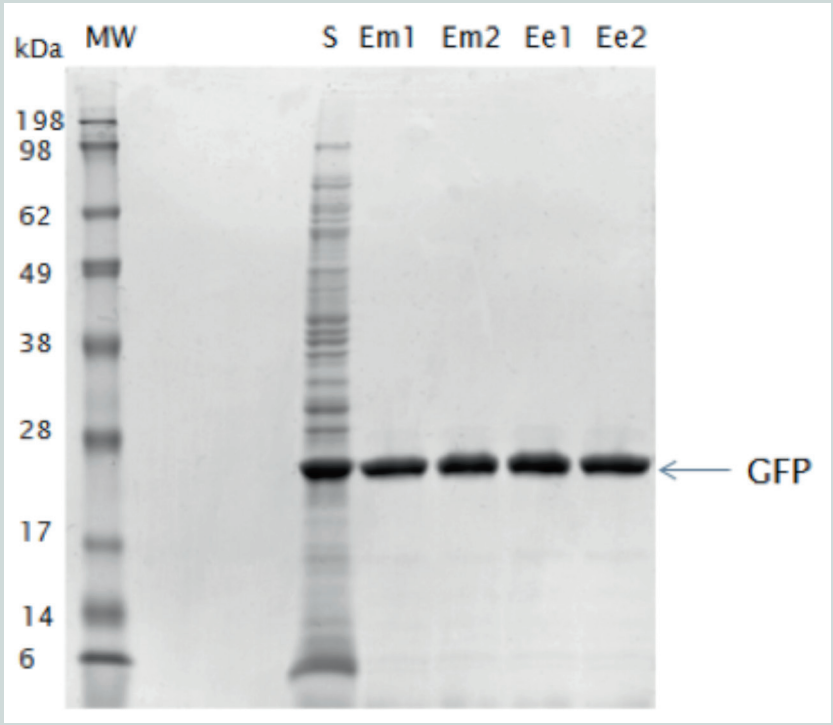

Figure 3: Comparison of manual (m) and automated (e) purification process on 2 replicates of E. *coli* lysate spiked with 100 µg GFP protein. 12 % SDS-PAGE gel analysis of 5 µL of sample before purification (S) and eluted fractions (E).

Ordering Information

Description	Order no. international
ep*Motion*® M5073	5073 000.205
TS 1000 single channel dispensing tool	5280 000.053
PrepRack for 24 Eppendorf Safe-Lock Tubes 2 mL	5075 751.006
Thermorack for 24 tubes, 1.5/2.0 mL	5075 771.004
ReservoirRack	5075 754.002
Reservoir Rack Module TC, for use in ep*Motion* Reservoir Racks, temperature-controlled, 2 x reaction vessels diameter 29 mm	5075 799.189
epT.I.P.S.® Motion 1000 µL with Filter	0030 014.499
Reservoir 100 mL	0030 126.513
Liquid Waste Tub	5075 751.500
Protein LoBind Tubes, 1.5 mL	0030 108.116
Protein LoBind Tubes, 2 mL	0030 108.132

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