VBCF – Protein Technologies	**Standard Operation Procedure** **Manual Magnetic Bead IMAC Screen Protocol**

1. **Aim:**
   Test expression in E. coli and purification using magnetic IMAC beads of target constructs.
2. **Applicability:**
   E. coli pellets in a 96 deep-well block
3. **Responsibilities:**
   Main Responsible: Anita Lehner
   Substitute: Orla Dunne, David Drechsel
4. **Required materials:**
   96-well deep-well plate (Greiner Bio-One 780270)
   Buffer NPI-10-Tween (see below)
   Buffer NPI-20-Tween (Wash buffer) (see below)
   Buffer NPI-250-Tween (see below)
   Lysozyme (Sigma L-6876)
   benzonase (MBS)
   flat-bottomed microtitre plate (Greiner 655101)
   8 M Urea buffered with 100 mM NaH2PO4 and 10 mM Tris to pH 8.0
   96-Well Magnet (QIAGEN 9014061 or 9012916)
   Promega Magne-His beads (V8560)
   4% formic acid
   SDS-PAGE sample buffer 6X
   96-well PCR plate (BIOZYM 710879)

Buffer NPI-10-Tween (50 mM NaH2PO4, 300 mM NaCl, 10 mM imidazole, 1% v/v Tween 20, adjust pH to 8.0 using NaOH and filter before use. Store at 4°C.)

Buffer NPI-20-Tween (Wash buffer, 50 mM NaH2PO4, 300 mM NaCl, 20 mM imidazole, 0.05% v/v Tween 20, adjust pH to 8.0 using NaOH and filter before use. Store at 4°C.)

Buffer NPI-250-Tween (50 ml, elution buffer, 50 mM NaH2PO4 , 300 mM NaCl, 250 mM Imidazole , 0.05% Tween , adjust pH to 8.0 using NaOH and filter before use. Store at 4°C.)

DNAse I Stock Solutions (40,000 units/ml, To a 200,000 unit bottle of DNAse I (Sigma D-4527) add 5ml of UHQ Sterile Water. Aliquot into 25µl aliquots store at -20°C. Add one aliquot to the 25ml of Buffer NPI-10-Tween buffer to be used for lysis.)

Lysozyme (Add 25mg of freshly-weighed Lysozyme (Sigma L-6876 or equivalent) to the 25ml of Buffer NPI-10-Tween buffer to be used for lysis.)

5. Procedure:

A. Preparatory work

This protocol starts with E.coli pellets from 2ml/well of a 96-well deep-well plate (e.g. Greiner Bio-One 780270) that have been harvested by centrifugation at ca. 5000g for 30 minutes at 4°C. The supernatants/media have been removed by inversion of the plate and the pellets frozen to -80°C (or on dry ice if more convenient) for at least 1 hour prior to screening (this aids lysis of the E.coli). We find 24-well plates are more appropriate for E.coli growth with 4-5ml/well volumes, these are then re-formatted from 4x24-well to 1x96 well plates for harvesting. Growing 4-5mls also allows a replica plate to be made that can be very useful.

B. Standard protocol for Qiagen BioRobot 8000

This manual protocol is adapted from that used on the QIAGEN BioRobot 8000 with QIAGEN Magnetic Ni-NTA beads* but can be easily performed with the aid of a multi-channel pipettor (MCP) and an appropriate orbital microplate shaker**. This is available as a standard protocol on the Qiagen BioRobot 8000 but must be specifically scripted for all other robotic platforms.

Step 1

Resuspend the cells completely in 210µl of Buffer NPI-10-Tween supplemented with 1mg/ml Lysozyme and either 3 units/ml of Benzonase*** (Merck, Germany; purity grade I, ≥ 25 U/µl, Cat. No. 1.01694.0001) or 400 units/ml of DNAse Type I. This can be done by either repeated aspirate/dispense with a suitable multi-channel pipette or on an orbital MTP shaker (~1000 r.p.m. for 30 minutes). Allow 30 minutes for the action of the Lysozyme and DNAse before clearing the lysate by centrifuging the deep-well block at 5000g for 30 minutes at 4°C (the Beckman JS5.3 rotor for the Beckman Avanti centrifuges is ideal for this).

Step 2

Approximately 5 minutes before the end of the centrifugation run dispense 20µl of the Ni-NTA magnetic bead suspension (ensure full re-suspension before you commence pipetting!) into each well of a flat-bottomed MTP. [Not all micro-titre plates are magnet-compatible! Check before you commence the assay, Greiner 655101 MTPs work well but many others have a continuous flat base that does not allow the magnets access to the spaces between the plate wells].

Step 3

Transfer the supernatants from Step 1 without disturbing the 'insoluble' pellets to each well of the MTP containing the Ni-NTA magnetic beads. Mix for 30 min at room temperature using either a MTP shaker or, alternatively, vortex at 600 rpm using an adapter for MTPs. The pellets may be re-suspended in 8 M Urea buffered with 100 mM NaH2PO4 and 10 mM Tris to pH 8.0 for analysis of the 'insoluble' fraction on SDS-PAGE.

Step 4

Place the 96-well microplate on the 96-Well for 1 min and remove the supernatant carefully from the beads with a MCP. [We find the QIAGEN magnets Type A or B, Qiagen part no.s 9014061 and 9012916 respectively, work well but there are many other suitable magnets on the market.]

Step 5

Add 200 µl of Wash Buffer to each well, remove from magnet and mix on the microplate shaker for 5 minutes.

Step 6

Place on the 96-Well Magnet for 1 min, and remove buffer.

Step 7

Repeat steps 5 and 6.

Step 8

Add 50 µl of Elution Buffer to each well, mix on the MTP shaker (or vortex) for 1 min, place on the 96-Well Magnet for 1 min, and transfer the supernatant (eluate) to a fresh PCR/MTP for analysis. [SDS-PAGE sample buffer may be used instead of elution buffer if you require a more concentrated sample for analysis. Elution may also be performed in up to 4% formic acid for direct use in mass spectrometry.]

Step 9

Load 5-15 µl on SDS-PAGE gels and stain with Coomassie OR run samples on Caliper or equivalent CE instrument.

Notes:

The addition of Tween 20 to the buffers is necessary to enable optimal collection of the magnetic beads on the sides of the MTP wells and also facilitates efficient cell lysis in step 1. Bead volumes etc. are based on the QIAgen protocol and may require adjustment if reagents from another supplier are used.

* We prefer to use 20 µl Promega Magne-His beads. When using these beads you may obtain cleaner results with using 20 mM Imidazole for lysis and 40 mM for washing (c.f. 10 and 20 mM with Qiagen beads). Promega beads also work with Urea (for us the Qiagen beads did not) and may have a longer shelf-life.

** We have successfully used two MTP shakers -BigBear automation do a good range of manual or PC/robot-controlled shakers with an orbit of 2mm (http://www.bigbearautomation.com/orbitalshaker.htm). Similarly Thermo/Inheco/VarioMag make the Teleshake manual or PC/robot-controlled shaker range but for us only the 3mm orbit shaker seemed up to the job of lysing the cells.

*** Benzonase is recommended as it is more stable than DNase I. When using Benzonase/DNaseI the crude lysate may be used directly in the binding step but the results will be less informative with regards to the solubility/cellular partitioning of the proteins.

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