GE Healthcare

The Trap platform and Tecan automation – efficient solutions for screening proteins

TECAN.

GE Healthcare and Tecan – working together to get it right from the start

Screening recombinant proteins in drug discovery and protein research is complex enough without the added concern of making different systems work together.

That's why GE Healthcare and Tecan are working together. With over 50 years of experience, we have recognized expertise in our respective fields of protein separation and laboratory automation. We are collaborating to provide robust solutions for protein expression screening, so that you can concentrate on making discoveries.

Welcome to new dimensions of quality and reliability in protein expression screening.

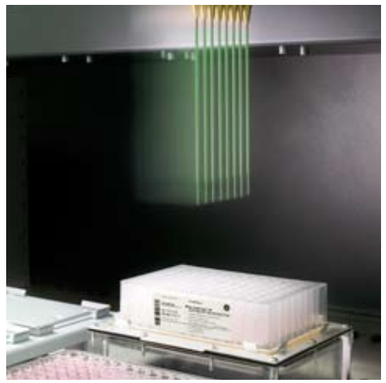
Automation – a key to success

The development of protocols for efficient approaches to cloning, expression, purification and crystallization of large numbers of tagged recombinant proteins is essential to shorten the time from gene to drug target. Automation increases throughput at each stage, and enables methods to be performed with a high level of robustness – increasing accaracy, enabling hands-off operation and eliminating human error – to give uncompromising experimental reproducibility and minimal variation among replicates.

This brochure highlights proven automated solutions for real challenges in protein expression screening, with examples that show how the combination of our products and expertise can help you.

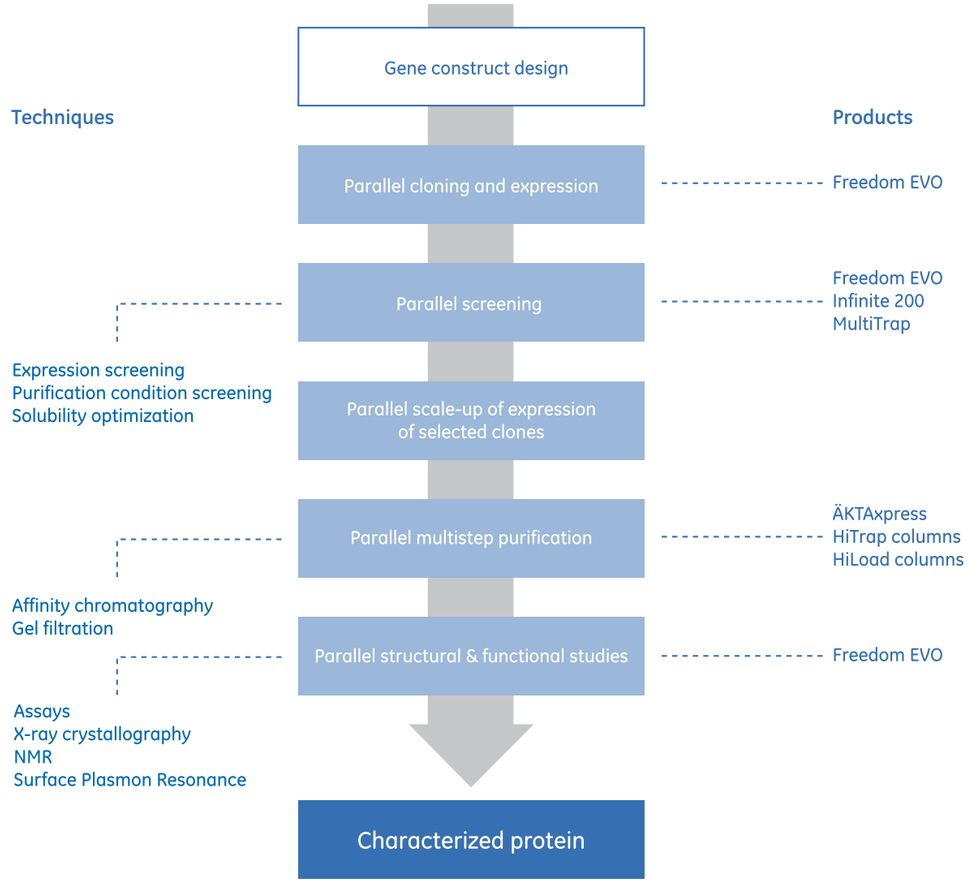
- Multi-parallel screening of tagged proteins with high reproducibility and minimal cross-contamination.
- Screening of buffer conditions to optimize the recovery of a target protein, and using the identified conditions to scale up the purification.
- Identification of protein construct variants that improve crystallization propensity.

For more information, please visit: www.gelifesciences.com/trap and www.tecan.com.



Freedom EVO and MultiTrap

From gene construct design to characterized protein



The workflow above is a typical example of a protein design process that requires a parallel approach to optimize protein constructs efficiently. Typical uses are for determining protein expression levels, solubility, yields, tag cleavage, optimal buffer conditions, homogeneity and purity. To make your work more efficient, we offer a range of products (see above) that are designed to work together for maximum performance and minimum variation among replicates.

MultiTrap for highly consistent results

MultiTrap™ from GE Healthcare provides three strong benefits for protein expression screening:

- Efficient parallel processing
- A high degree of reproducibility
- Low cross-contamination among samples

MultiTrap is a ready-to-use, prepacked 96-well filter plate that simplifies screening and small-scale parallel purification and secures consistent results with high reproducibility, both well-to-well and plate-to-plate. MultiTrap is suitable for simultaneously measuring expression levels of different constructs and expression systems, as well as for optimizing screening parameters to improve, for example, protein solubility, recovery and purity.

MultiTrap 96-well filter plate

MultiTrap can be loaded with up to 600 µl of sample in each well, giving microgram to low milligram amounts of enriched protein. MultiTrap accommodates both centrifugation and vacuum protocols and requires less sample preparation time, since unclarified samples can be loaded directly (Figure 1).

His MultiTrap and GST MultiTrap filter plates are prepacked with Ni Sepharose™ or Glutathione Sepharose for efficient high-throughput, multi-parallel screening of polyhistidine- and glutathione S-transferase (GST)-tagged proteins. Ni Sepharose media for binding histidine-tagged proteins are characterized by negligible leakage of nickel ions, high protein binding capacity and chemical stability with a wide range of reagents, making them highly suitable for screening additives and buffers. Two chromatography media are available for MultiTrap: Ni Sepharose High Performance (34 µm beads) and Ni Sepharose 6 Fast Flow (90 µm beads). Applications described in this brochure feature histidine-tagged proteins but the methods are applicable for most tags and proteins.

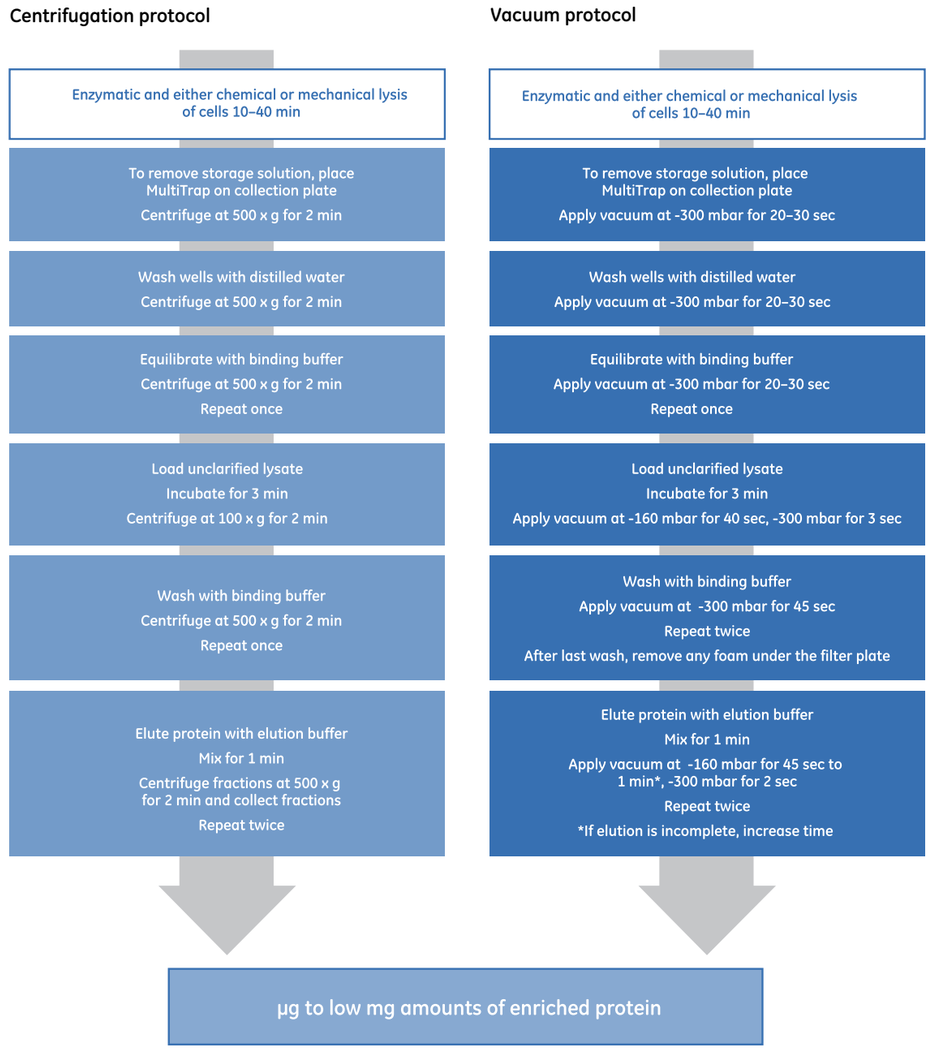


Fig 1. Centrifugation protocol (left) for purifying tagged proteins using MultiTrap. A typical automated method is executed in 60 minutes. Vacuum protocol (right) for purifying tagged proteins using MultiTrap. A typical automated method is executed within one hour.

As well as for tagged protein applications, MultiTrap products are available for:

- Antibody purification
- Protein enrichment by immuno-affinity
- Desalting, buffer exchange, and cleanup of proteins and carbohydrates

For more information, please visit www.gelifesciences.com/trap.

Automated solutions for screening and purification

Freedom EVO – flexible automation

Freedom EVO® is a flexible liquid handling workstation, to which a range of modules can be easily integrated for the automation of recombinant protein screening and purification steps, including:

- Cell transformation, cell lysis and sample preparation
- Purification using Te-VacS™ vacuum separation module
- Centrifugation
- Result analysis using Infinite™ 200
- Protein crystallization

Freedom EVO

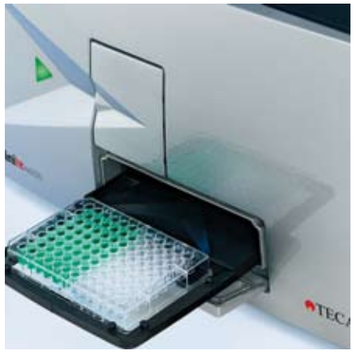
Freedom EVO and MultiTrap – high quality partners

The combination of Tecan Freedom EVO and GE Healthcare MultiTrap gives reliable, automated, parallel, one-step purification of tagged proteins from plate preparation, sample application, washing and elution, through to analysis.

For more information, please visit www.tecan.com

Infinite 200 – flexible detection for all applications

The Infinite 200 series of microplate readers offers all main detection technologies for 6- to 384-well plates, PCR plates and cuvettes in one easy-to-use instrument. Advanced optics for fluorescence, luminescence and absorbance provide outstanding flexibility for all life science applications. The Infinite 200 series uses quad4 monochromators™ technology for excellent sensitivity, or a patented filter system for routine applications.



Infinite 200

ÄKTAxpress – unattended protein purification

Once conditions are optimized with MultiTrap and Freedom EVO, protein purification, scale-up is simple using ÄKTAxpress™. Fully automated multistep purification of up to four different affinity tagged proteins are achieved in one run, one per module. Milligram amounts of highly pure protein are obtained. For increased throughput, up to 12 different ÄKTAxpress modules can be controlled in parallel by one computer.

ÄKTAxpressTWIN

High reproducibility

Combining Freedom EVO and MultiTrap ensures high reproducibility. To demonstrate this, unclarified *E. coli* lysates containing histidine-tagged green fluorescence protein ($(His)_6$-GFP, M_r 28 000) and histidine-tagged maltose binding protein ($(His)_6$-MBP, M_r 43 800) were applied in a chessboard pattern across His MultiTrap HP and purified automatically on Freedom EVO equipped with Te-VacS vacuum separation module. Protein concentration was measured by Infinite 200 at 280 nm.

Figure 3 shows the yield of $(His)_6$-GFP from His MultiTrap HP. The results show good well-to-well reproducibility in the yield of target proteins, with low relative standard deviations (RSD). The RSD of the yield of purified $(His)_6$-MBP was similar (data not shown).

Te-VacS vacuum separation module

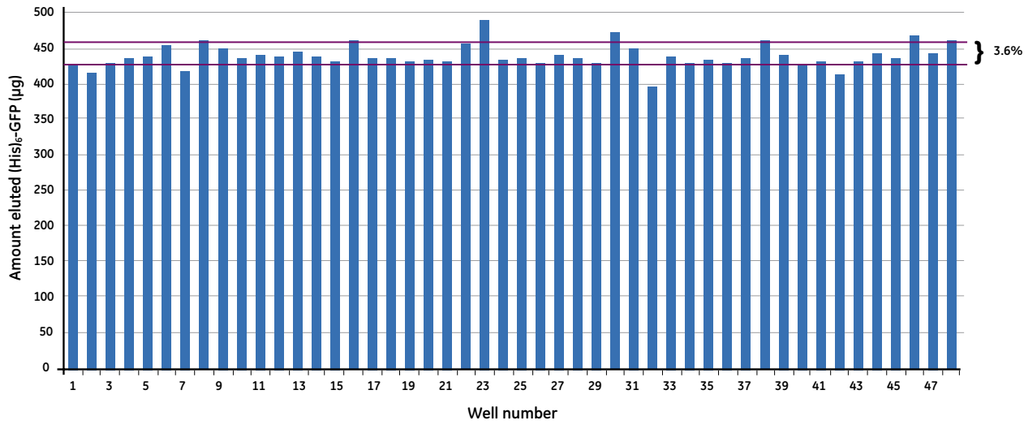


Fig 2. Reproducibility in the yield of purified $(His)_6$-GFP from the first elution. Replicate runs showed a relative standard deviation of 3.6% in the amount of protein recovered in the first elution. The average yield from the first elution was 441 µg. A second elution can add as much as 25% to the total recovery of protein (an additional 120 µg in this example).

Summary

Tecan automation and GE Healthcare MultiTrap give consistent well-to-well performance, so that detected differences can be attributed to the protein constructs.

Screening of buffer conditions

Ninety-six buffer conditions were simultaneously screened to find optimum purification conditions of expressed target protein by a parallel, fully automated, one-step purification approach on a single MultiTrap filter plate, using Tecan Freedom EVO liquid handling workstation and a centrifuge.

Clarified *E. coli* lysates containing histidine-tagged Nurr1 ligand binding domain (His-Nurr1-LBD) (M_r 26 000), a transcription factor crucial for the development of dopamine neurons, was applied to His MultiTrap FF. Eight buffer solutions with varying pH ranging from 6.0 to 8.5 were screened (Table 1). For each of the eight buffers, 12 buffer solutions with varying NaCl, glycerol and ß-mercaptoethanol were screened (Table 2). Binding buffer contained 50 mM imidazole, and elution buffer contained 500 mM imidazole.

SDS-PAGE was used to determine the recovery of the target protein from the lysate (Fig. 3).

Table 1. Buffers tested at a range of pH

25 mM MES, pH 6.0	25 mM HEPES, pH 7.5
25 mM PIPES, pH 6.5	25 mM HEPES, pH 8.0
25 mM sodium phosphate, pH 7.0	25 mM Tris, pH 8.0
25 mM sodium phosphate, pH 7.5	25 mM Tris, pH 8.5

Buffer screening

Table 2. Concentrations of additives in the buffer

	1	2	3	4	5	6	7	8	9	10	11	12
NaCl (mM)	100	200	300	400	500	750	100	200	100	200	100	200
Glycerol (%)							5	5	10	10	10	10
ß-mercaptoethanol (%)											0.05	0.05

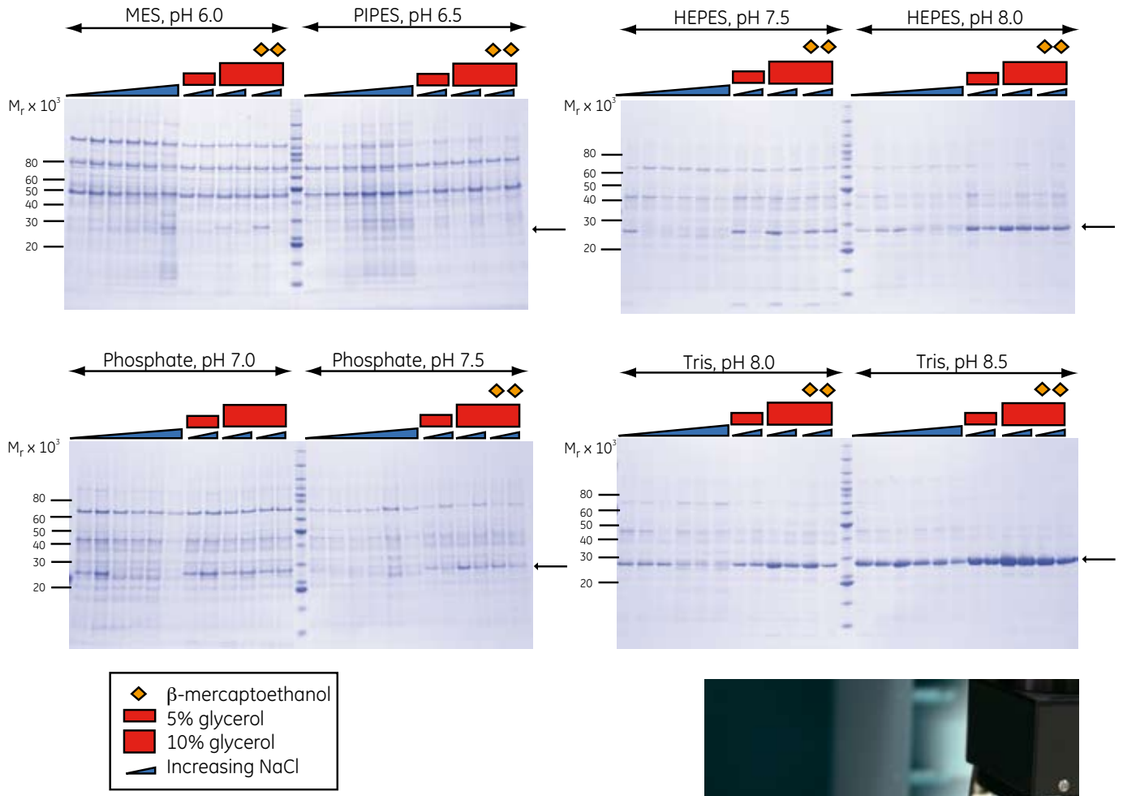
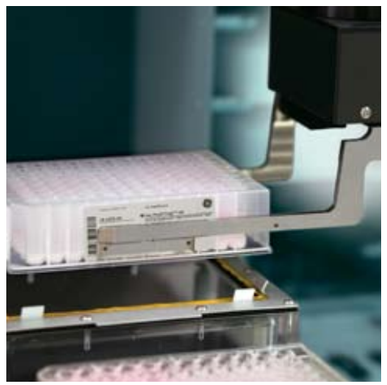


Fig 3.
Coomassie™ stained SDS-PAGE analysis of eluted His-Nurr1-LBD. For each of eight buffers (Table 1), 12 buffer compositions were analyzed, with varying concentrations of NaCl, glycerol and ß-mercaptoethanol (Table 2). The arrows indicate the position of target protein, His-Nurr1-LBD. The highest amount of purified protein was recovered in the buffer at pH 8.5 (25 mM Tris, 100 mM NaCl, 10% glycerol, pH 8.5).



MultiTrap and Freedom EVO

Summary

Buffer screening using MultiTrap and Freedom EVO shortens the process of finding optimal conditions for solubility.

Scaling up protein production

Using the optimal conditions established by buffer screening, purification of the target protein was scaled up 20 times from 50 µl to 1 ml, from His MultiTrap FF to HisTrap™ FF, 1ml (Fig 4A). The second purification step was carried out with the prepacked gel filtration column HiLoad™ 16/60 Superdex™ 200 pg (Fig 4B).

ÄKTAxpress system was programmed to run a two-step purification. High purity target protein was obtained, and a mixture of monomer, dimer and trimer could be observed in the eluted peaks from the gel filtration column under the buffer conditions used.

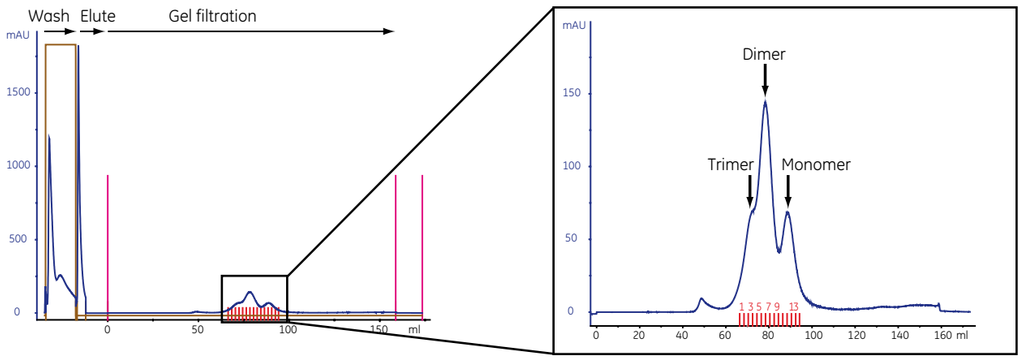


Fig 4A. Chromatogram from the entire run shows the first IMAC step with HisTrap FF and the second gel filtration step with HiLoad 16/60 Superdex 200 pg.

Fig 4B Zoom of the second purification step performed on ÄKTAxpress using HiLoad 16/60 Superdex 200 pg.

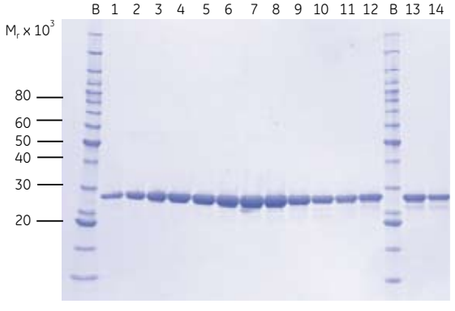


Fig 5. SDS-PAGE (Coomassie staining) purity analysis of peak fractions collected from the second gel filtration purification step (reduced conditions). B: molecular weight markers.

HisTrap

Summary

Screening of expression and purification conditions secures successful results when scaling up the purification.

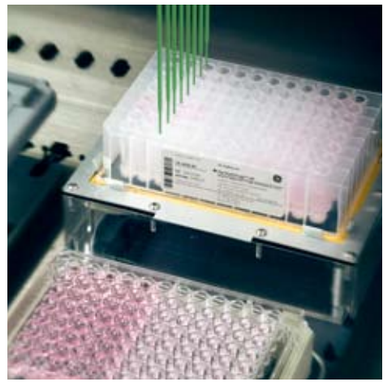
Acknowledgments: Ruth Steele and Dr Bruce Grasberger, Johnson and Johnson, Exton, USA

Minimal cross-contamination

The combination of automation and vacuum control protocols ensures minimal contamination between wells. Clarified *E. coli* lysate containing histidine-tagged enhanced green fluorescent protein ($(His)_6$-EGFP, M_r 28 000) and histidine-tagged kinase ($(His)_6$-kinase, M_r 43 800) were applied in a chessboard pattern across one His MultiTrap HP plate (Fig 6A). Eluates from each well were analyzed by SDS-PAGE. The entire protocol was carried out using a Tecan liquid handling workstation and the Te-VacS vacuum separation module. No significant cross-contamination was seen in SDS-PAGE analysis (Fig 6B).

	1	2	3	4	5	6	7	8	9	10	11	12
A	K	E	K	E	K	E	K	E	K	E	K	E
B	E	K	E	K	E	K	E	K	E	K	E	K
C	K	E	K	E	K	E	K	E	K	E	K	E
D	E	K	E	K	E	K	E	K	E	K	E	K
E	K	E	K	E	K	E	K	E	K	E	K	E
F	E	K	E	K	E	K	E	K	E	K	E	K
G	K	E	K	E	K	E	K	E	K	E	K	E
H	E	K	E	K	E	K	E	K	E	K	E	K

Fig 6A. Chessboard sample application scheme.
K: $(His)_6$-kinase
E: $(His)_6$-EGFP



His MultiTrap HP in Te-Vacs vacuum separation module

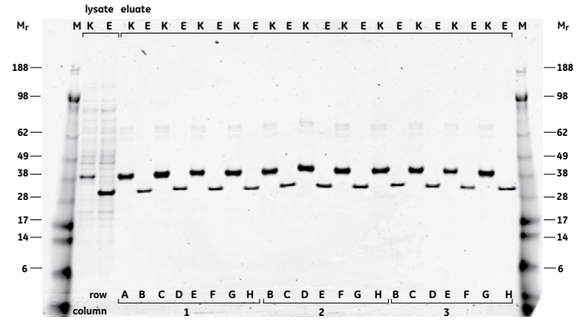


Fig 6B. Coomassie-stained SDS-PAGE analysis (reduced conditions) of purified $(His)_6$-kinase and $(His)_6$-EGFP using His MultiTrap HP.
Lanes:
M: molecular weight markers
K: $(His)_6$-kinase, M_r 43 800) in lysate and eluates of 12 wells
E: $(His)_6$-EGFP, M_r 28 000) in lysate and eluates of 12 wells

Summary

"The apparent absence of cross-contamination between GFP and kinase was further analyzed by fluorescence. This more sensitive read-out demonstrated the reliability of the system, as there is at least a 20-fold difference in signal between the two sets. MultiTrap plates on a Tecan system are a winning combination."

Dr. Arndt Schmitz, Bayer Schering Pharma AG, Berlin, Germany.

Acknowledgments: Mario Mann, Dr Guido Malawski and Dr Arndt Schmitz, Bayer Schering Pharma AG, Berlin, Germany

High-throughput expression screening and purification

MAPKAP kinase 2 (MK2, M_r 38 000) is a key enzyme in the inflammation pathway and is therefore an attractive drug target for cancer therapy. A large set of variants and mutants of MK2 were expressed with histidine tags and screened for expression and purification.

High-throughput expression screening
Expression levels of 24 different histidine-tagged truncated variants of kinase were expressed in a transient insect cell system (to obtain post-translational modifications) and screened to purify the proteins for further crystallization studies. All 24 variants were purified in parallel using His MultiTrap HP and the Tecan liquid handling workstation with the vacuum protocol, and the eluted fractions were analyzed by SDS-PAGE (Fig 7).

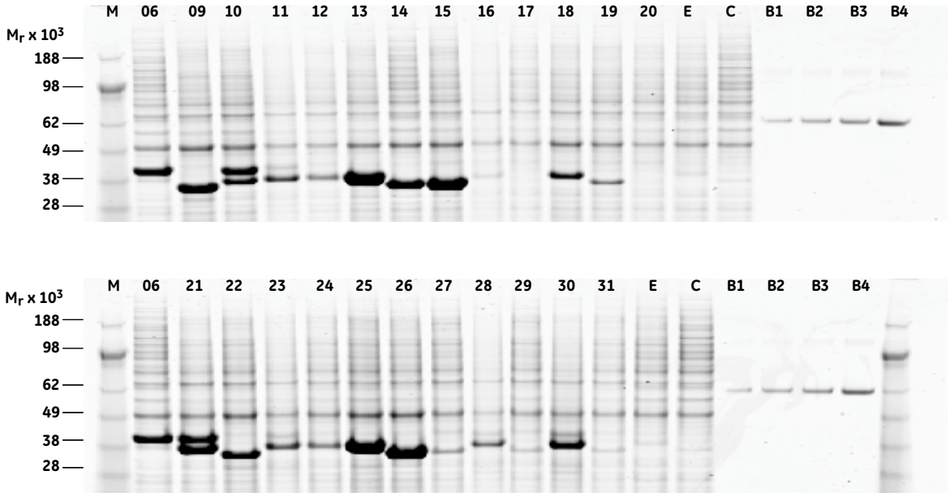


Fig 7. SDS-PAGE analysis of eluates of histidine-tagged truncated variants of kinase, expressed in transient insect cell system for 72 h. Lanes 06-31: histidine-tagged variants of kinase, E: enhanced green fluorescent protein (untagged, transfection positive control), C: non-transfected Hi5 cells, B1 to B4: 0.5, 0.75, 1.0 and 1.5 µg BSA respectively.

Summary
Use of MultiTrap with Tecan automation for parallel screening of expression improved project timeliness and the success of subsequent steps in the workflow, such as X-ray crystallographic studies.

Reference
Malawski G. *et al.* Identifying protein construct variants with increased crystallization propensity – A case study. Protein Science 15, 2718–2728 (2006).

Acknowledgments: Mario Mann, Dr. Guido Malawski and Dr. Arndt Schmitz, Bayer Schering Pharma AG, Berlin, Germany.

Ordering information

Product	Quantity	Code No.
His MultiTrap FF	4 × 96 well plates	28-4009-89
His MultiTrap HP	4 × 96 well plates	28-4009-90
GST MultiTrap 4B	4 × 96 well plates	28-4055-00
GST MultiTrap FF	4 × 96 well plates	28-4055-01
PD MultiTrap G-25	4 × 96 well plates	28-9180-06
Protein G HP MultiTrap	4 × 96 well plates	28-9031-33
Protein A HP MultiTrap	4 × 96 well plates	28-9031-35
Streptavidin HP MultiTrap	4 × 96 well plates	28-9031-31
Collection plate, 500 µl V-bottom	5 × 96 well plates	28-4039-43
ÄKTAxpress TWIN	Chromatography system	11-0012-84
ÄKTAxpress module for system extension	Chromatography system	18-6645-01

www.gelifesciences.com/trap
www.gelifesciences.com/protein-purification for more information about HiTrap™, HiPrep™, HiLoad prepacked columns and other chromatography systems.

Freedom EVO – specifications

General hardware features	
Robotic arms	Liquid handling arms, robotic manipulators, several multi-channel options
Tip configuration	1, 2, 4, 8 tips, various combinations of application-oriented tip types
Tip types	Standard (Teflon™-coated stainless steel) and disposable tips with or without filter (10/200/1000 µl); low-volume tips for high-density format applications
Syringe sizes	25/50/250/500/1000/2500/5000 µl

Liquid handling features				
Volume range	0.5 µl-5000 µl			
Pipetting precision	Volume	Standard tips	Disposable tips	
			200 µl	1000 µl
	10 µl	≤ 3.0%	≤ 3.0%	-
	100 µl	≤ 0.5%	≤ 0.5%	≤ 1.0%

www.tecan.com for more information about Tecan Freedom EVO platforms, Te-VacS and Tecan readers such as Infinite 200.

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala
Sweden

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

Tecan
Austria
+43 62 46 89 33

Belgium
+32 15 42 13 19

China
+86 10 5869 5936

Denmark
+45 70 23 44 50

France
+33 4 72 76 04 80

Germany
+49 79 51 94 170

Italy
+39 02 92 44 790

Japan
+81 44 556 73 11

Netherlands
+31 18 34 48 174

Portugal
+351 21 000 82 16

Singapore
+65 644 41 886

Spain
+34 93 490 01 74

Sweden
+46 31 75 44 000

Switzerland
+41 44 922 89 22

UK
+44 118 9300 300

USA
+1 919 361 5200

ROW
+43 62 46 89 33

For contact information for your local office, please visit: www.gelifesciences.com/contact

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

www.gelifesciences.com

imagination at work

For contact information for your local office, please visit: www.tecan.com

Tecan Switzerland
Seestrasse 103
8408 Männedorf ZH
Switzerland

www.tecan.com

TECAN.

28-9289-59AA 10/2007