GST MultiTrap FF
GST MultiTrap 4B

GST MultiTrap™ FF and GST MultiTrap 4B are prepacked, 96-well filter plates for reproducible, high-throughput screening and rapid parallel purification of glutathione S-transferase (GST) tagged proteins. Purification of up to 0.5 mg of GST-tagged proteins/well directly from unclarified cell lysate can be achieved using GST MultiTrap 96-well filter plates. Loading unclarified lysate directly to the wells shortens handling time and minimizes degradation of sensitive target proteins. The 96-well plate format gives the choice of using centrifugation or vacuum, automated in robotic systems or manually operated. Consistent well-to-well and plate-to-plate performance ensures high reproducibility.

Prepacked GST MultiTrap 96-well filter plates offer:

- Highly reproducible well-to well and plate-to-plate results
- High purity in one step
- High chemical stability
- Convenience: wells prepacked with Glutathione Sepharose™ media eliminate the need for plate filling
- Simplified workflow for screening: load unclarified cell lysates directly to the wells
- Easy and predictable scale-up using prepacked GSTrap™ FF, GSTPrep™ FF 16/10, and GSTrap 4B columns

96-well filter plates

GST MultiTrap 96-well filter plates (Fig 1) are manufactured from biocompatible polypropylene and polyethylene material. Plates are bar coded for easy and reliable sample tracking. Each well has a volume of 800 µl, prepacked with 50-µl medium in 20% ethanol (500 µl solution of 10% slurry). GST MultiTrap 96-well filter plates give simple, yet highly reproducible purifications of up to 0.5 mg GST-tagged protein/well. Plates are supplied in packs of four and are sealed at the top and bottom.

Fig 1. GST MultiTrap FF and GST MultiTrap 4B are prepacked 96-well filter plates for highly reproducible screening and parallel purification of GST-tagged proteins from unclarified or clarified samples.

GST MultiTrap FF and GST MultiTrap 4B 96-well filter plates allow parallel purification of GST-tagged proteins directly from unclarified cell lysates. No centrifugation or filtration is needed before loading the sample onto the filter plate. GST MultiTrap filter plates enable a short purification time, which minimizes degradation and oxidation of sensitive target proteins.

Table 1 shows the characteristics of GST MultiTrap 96-well filter plates.

Prepacked media

GST MultiTrap FF and GST MultiTrap 4B 96-well filter plates are prepacked with Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose 4B media, respectively. These media consist of 90-µm beads of spherical agarose to which a glutathione ligand has been coupled (Table 1). The agarose beads of Glutathione Sepharose 4 Fast Flow are highly cross-linked while those of Glutathione Sepharose 4B are not cross-linked. Coupling is optimized to give high binding capacity for GST-tagged proteins and other glutathione-

Table 1. Characteristics of GST MultiTrap FF and GST MultiTrap 4B

Filter plate size[1]	127.8 × 85.5 × 30.6 mm according to ANSI/SBS 1-2004, 3-2004 & 4-2004 standards
Filter plate material	Polypropylene and polyethylene
Media	GST MultiTrap FF: Glutathione Sepharose 4 Fast Flow; highly cross-linked, spherical agarose, 4%, with a glutathione ligand coupled via a 10-carbon linker arm. GST MultiTrap 4B: Glutathione Sepharose 4B; 4% spherical agarose, with a glutathione ligand coupled via a 10-carbon linker arm.
Average particle size	90 µm
Binding capacity[2]	Up to 0.5 mg GST-tagged protein/well
Recommended elution conditions	10 to 30 mM reduced glutathione in elution buffer
Reproducibility between wells	Amount of eluted target proteins/well does not differ more than ± 10% from the average amount/well for the whole filter plate
Volume packed medium/well	50 µl (500 µl of 10% slurry)
Well volumes	800 µl
Centrifugation speed[3]	
Recommended	100 to 500 × g
Maximum	700 × g
Vacuum pressure[3]	
Recommended	–0.1 to –0.3 bar
Maximum	–0.5 bar
Chemical stability	All commonly used aqueous buffers, e.g. 1 M acetate, pH 4.0 and 6 M guanidine hydrochloride for 1 h at room temperature.
pH stability	3–12 (Glutathione Sepharose 4 Fast Flow), 4–13 (Glutathione Sepharose 4B)
Storage solution	20% ethanol
Storage temperature	4°C to 30°C (GST MultiTrap FF) 4°C to 8°C (GST MultiTrap 4B)

[1] ANSI = American National Standards Institute. SBS = Society for Biomolecular Screening

[2] Binding of GST-tagged protein depends on size, conformation, and concentration of the protein in the sample loaded

[3] Depends on sample pretreatment and sample properties

GST-tagged proteins expressed using, for example, pGEX expression vectors, can be purified directly from unclarified bacterial lysates. GST-tagged proteins are eluted under mild, nondenaturing conditions that preserve protein antigenicity and function. Purification can be easily scaled up using GSTrap FF, GSTPrep FF 16/10, or GSTrap 4B columns.

Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose 4B media are compatible with a wide range of detergents, reducing agents, and other compounds used in protein screening purification (Table 2).

Operation with robotic systems, centrifugation, or vacuum

The filter plates can be used with a robotic system or operated manually by centrifugation or vacuum. A general sample preparation protocol involves: suspending the cells/cell paste; enzymatic lysis using lysozyme, DNAse I, and adding $MgCl_2$ etc; mechanical lysis by sonication, homogenization, freeze/thaw or lysis using a commercially available lysis kit; adjusting pH and applying unclarified lysate directly to the wells. The procedure shown in Figure 2 describes a centrifugation-based protocol for purification of GST-tagged proteins.

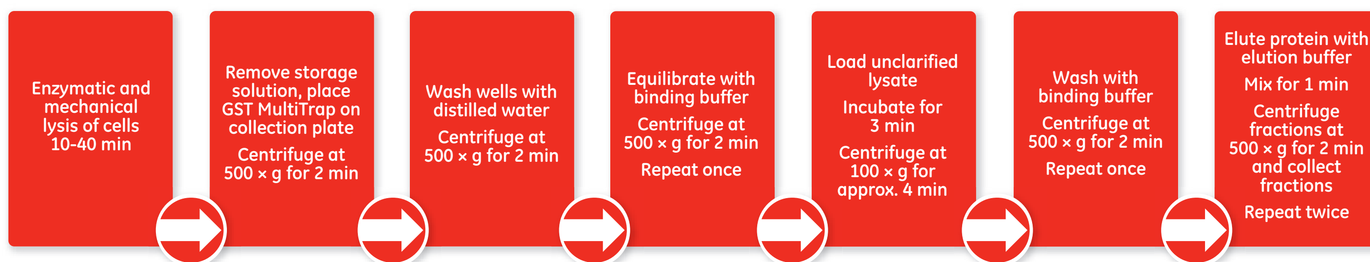


Fig 2. Protocol for purifying GST-tagged proteins by centrifugation using GST MultiTrap 96-well filter plates. A typical purification can be achieved in approximately 60 min. Refer to GST MultiTrap FF and GST MultiTrap 4B Instructions, 28-4070-75, for the full protocols.

Table 2. Glutathione Sepharose 4 Fast Flow and Glutathione Sepharose 4B are compatible with the following compounds at the concentrations given

Compound	Concentration
Reducing agents	5 mM DTE
	20 mM DTT[1]
	20 mM β-mercaptoethanol
	5 mM TCEP
	40 mM reduced glutathione
Denaturing agents[2]	8 M urea
	6 M guanidine-HCl
Detergents	2% Triton™ X-100 (nonionic)
	2% Tween™ 20 (nonionic)
	2% NP-40 (nonionic)
	2% cholate (anionic)
	1% CHAPS (zwitterionic)
Other additives	20% ethanol
	50% glycerol
	100 mM Na_2SO_4
	1.5 M NaCl
Buffers[3]	50 mM sodium phosphate
	100 mM Tris-HCl
	100 mM Tris-acetate
	100 mM HEPES
	100 mM MOPS

[1] DTT can improve binding of GST-tagged proteins and can be added to binding and elution buffers. A final concentration of 1 to 20 mM DTT is recommended.

[2] Denaturing agents are compatible with the media. However, the GST tag would be denatured using 6 M guanidine-HCl or 8 M urea and no protein will bind.. Lower concentrations may be used, but have to be optimized before the run since binding capacity may otherwise decrease compared to using native conditions.

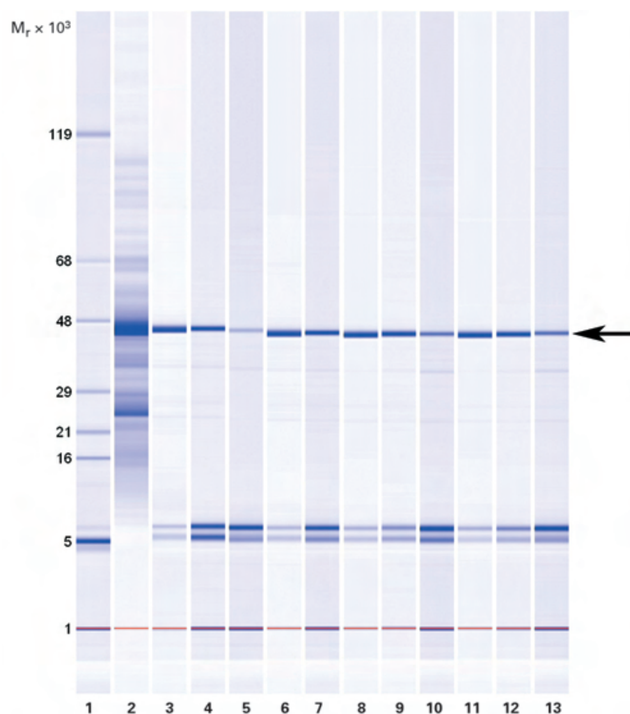
[3] pH 6.2 to 8.0.

Table 3. Reproducibility in purifying GST-hippocalcin on GST MultiTrap 96-well filter plates

	GST MultiTrap FF		GST MultiTrap 4B	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Plate-to-plate average yield of eluted GST-hippocalcin (µg)	408	407	465	469
Average difference in yield between plates (%)	0.5	0.5	0.9	0.9
Well-to-well RSD* (%)	5	5	5	5

* Relative standard deviation

96-well filter plates: GST MultiTrap FF and GST MultiTrap 4B
Sample: *E. coli* BL21 lysate containing GST-tagged hippocalcin, M_r 45 000
Sample preparation: Chemical lysis and sonication
Sample volume: 200 µl
Binding buffer: 10 mM sodium phosphate, 140 mM NaCl, pH 7.4
Elution buffer: 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Elution method: Centrifugation
Data evaluation: LabChip 90 Automated Capillary Electrophoresis System



Lanes
1. Molecular weight markers
2. Start material
3. GST MultiTrap FF: Eluent 1, replicate 1
4. GST MultiTrap FF: Eluent 2, replicate 1
5. GST MultiTrap FF: Eluent 3, replicate 1
6. GST MultiTrap FF: Eluent 1, replicate 2
7. GST MultiTrap FF: Eluent 2, replicate 2
8. GST MultiTrap 4B: Eluent 1, replicate 1
9. GST MultiTrap 4B: Eluent 2, replicate 1
10. GST MultiTrap 4B: Eluent 3, replicate 1
11. GST MultiTrap 4B: Eluent 1, replicate 2
12. GST MultiTrap 4B: Eluent 2, replicate 2
13. GST MultiTrap 4B: Eluent 3 replicate 2

Fig 3. Conditions used for purifying GST-tagged hippocalcin on GST MultiTrap plates and purity of recovered target protein. High purity of GST-hippocalcin purified was observed (arrow indicates the target protein).

High well-to-well and plate-to-plate reproducibility

Well-to-well and plate-to-plate reproducibility of GST MultiTrap FF and GST MultiTrap 4B plates in purifying unclarified GST-tagged hippocalcin was determined. *E. coli* containing GST-hippocalcin was chemically lysed and sonicated prior to purification on GST MultiTrap filter plates. Preparation of the filter plates and the purification protocol are described in Figure 2. Each purification performed on the 96-well plates was duplicated and three collections of eluent were made. Buffer conditions used in the purification are described in Figure 3. Yield was determined from collected eluent volumes. Protein purity was determined using LabChip™ 90 Automated Capillary Electrophoresis System.

Both GST MultiTrap FF and GST MultiTrap 4B show high well-to-well (relative standard deviation, 5%) and plate-to-plate (average yield differs by less than 1%) reproducibility in repeated runs (Table 3). The average yield for GST-hippocalcin was 15% higher using GST MultiTrap 4B compared to GST MultiTrap FF. However, yield is very protein

dependent for GST-tagged proteins and the choice of GST MultiTrap FF or GST MultiTrap 4B has to be investigated for individual GST-tagged proteins to obtain the highest yield.

High purity of GST-hippocalcin was obtained by purification on both GST MultiTrap FF and GST MultiTrap 4B plates (Fig 3).

Effect of different incubation times

The effect of different incubation times of GST-tagged protein sample on GST MultiTrap filter plates was investigated. *E. coli* containing GST-hippocalcin was chemically lysed and sonicated prior to purification. Unclarified lysate was applied to GST MultiTrap wells. The general purification protocol described in Figure 2 was employed, except that all centrifugation steps were performed at 100 × g. In addition, unclarified sample was incubated for 0, 3, 10, 30, 60, and 120 min.

After incubation and wash, samples were eluted and the effect of incubation time (Fig 4) evaluated. The yield of GST-hippocalcin reached a maximum at 15 min incubation, after which a small decrease was observed (Fig 4A). After only 3 min incubation on both GST MultiTrap FF and GST MultiTrap 4B filter plates, yield was greater than 90% of its maximum. Based on these results, optimal incubation time is between 3 and 15 min for GST-hippocalcin.

Purity of recovered GST-hippocalcin was high, as determined by SDS-PAGE (Fig 4B shows the purity for GST MultiTrap 4B; the purity of GST MultiTrap FF is not shown).

Easy scale-up with reliable results

Scaling up from GST MultiTrap FF or GST MultiTrap 4B filter plates to columns prepacked with the same medium is simple and reliable. Glutathione Sepharose 4 Fast Flow (prepacked in GST MultiTrap FF) is available in larger prepacked formats as GSTrap FF 1-ml and 5-ml columns, as well as GSTPrep FF 16/10 20-ml column. Glutathione Sepharose 4B (prepacked in GST MultiTrap 4B) is available in GSTrap 4B 1-ml and 5-ml columns. These columns are designed for syringe, peristaltic pump, or ÄKTAdesign™ chromatography systems such as ÄKTAxpress™. As the same buffer conditions apply to GST MultiTrap filter plates, GSTrap, and GSTPrep columns alike, highly consistent scale-up can be achieved with a minimum of optimization time.

Application

Buffer screening study

The aim of this work was to optimize binding buffer conditions for purification of GST-hippocalcin using GST MultiTrap FF. A buffer screening study to determine optimal buffer conditions for purification was designed based on the parameters buffer, pH, sodium chloride, glycerol, DTT and glutathione (Fig 5). A comparison between sonication and the use of a cell lysis kit, CelLytic™ B Plus Kit (Sigma-Aldrich), for the lysis of *E. coli*, was also performed. The experimental design was determined and statistical analysis performed

96-well filter plates:	GST MultiTrap FF and GST MultiTrap 4B
Sample:	*E. coli* BL21 lysate containing GST-tagged hippocalcin, M_r 45 000
Sample preparation:	Chemical lysis and sonication
Sample volume:	300 µl
Binding buffer:	10 mM sodium phosphate, 140 mM NaCl, pH 8.0
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Elution method:	Centrifugation
Data evaluation:	UV-spectrometry (A_{280}), SDS-PAGE

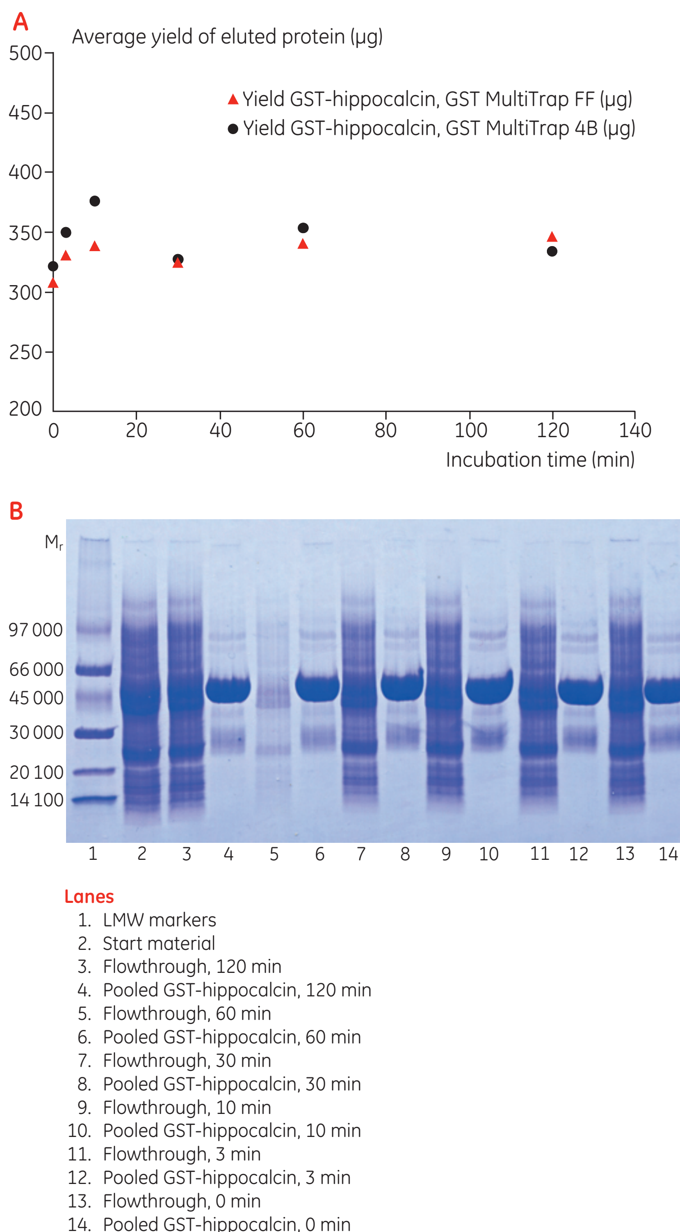


Fig 4. (A) Optimal incubation time of GST-hippocalcin on GST MultiTrap FF and GST MultiTrap 4B is between 3 and 15 min. (B) SDS-PAGE (ExcelGel™ SDS gradient 8–18, Multiphor™ Electophoresis System) of collected fractions from purification with GST MultiTrap 4B reveals pure GST-hippocalcin (M_r 45 000) is obtained irrespective of incubation time.

using MODDE software (Umetrics). The different buffer conditions and sample preparation methods were applied randomly on the filter plate.

Some of the results from the buffer screening/lysis method study are described in Table 4. The responses measured were yield (µg) and purity (%) of eluted GST-hippocalcin.

Table 4. Some results from a screening study using different binding buffer conditions (including sample and wash buffer) for purifying GST-hippocalcin on a GST MultiTrap FF 96-well filter plate. Yield and purity of eluted GST-hippocalcin are shown (32 different purification conditions and three replicates of each).

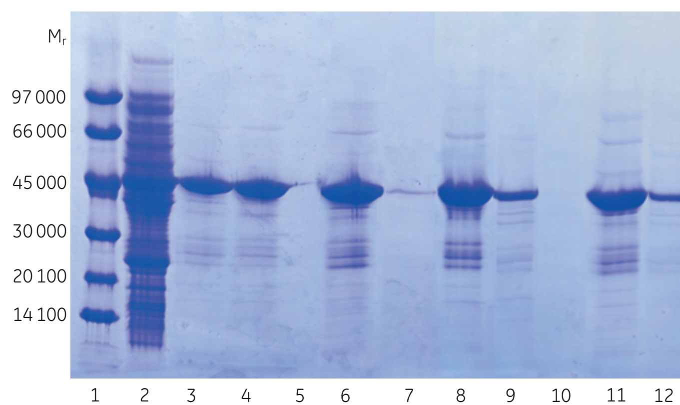
Binding buffer	Lysis method	Yield (µg)	Purity (%)	Lane in SDS-PAGE, Fig 5.
10 mM PBS, 140 mM NaCl, pH 7.4	Sonication	181	95	3
10 mM PBS, 140 mM NaCl, pH 7.4	Lysis using CelLytic B Plus Kit	191	94	4
10 mM PBS, 400 mM NaCl, 2 mM glutathione, 5% glycerol, pH 8	Lysis using CelLytic B Plus Kit	10	95	5
20 mM PBS, 400 mM NaCl, 5% glycerol, pH 6.2	Sonication	328	87	6
20 mM PBS, 400 mM NaCl, 2 mM glutathione, pH 8	Sonication	18	92	7
50 mM Tris-HCl, 400 mM NaCl, 5% glycerol, pH 6.2	Sonication	269	87	8
50 mM Tris-HCl, pH 8	Sonication	54	83	9
50 mM Tris-HCl, 140 mM NaCl, 2 mM glutathione, 5 mM DTT, 5% glycerol, pH 8	Sonication	–	–	10
100 mM Tris-HCl, 140 mM NaCl, 5 mM DTT, pH 6.2	Sonication	243	77	11
100 mM Tris-HCl, 270 mM NaCl, 1 mM glutathione, 2.5 mM DTT, 2.5% glycerol, pH 7.4	Sonication	164	91	12

The presence of glutathione in sample and binding buffer (also used as wash buffer) decreased yield of purified GST-hippocalcin significantly, while the type of buffer used had no adverse affect on yield. Low pH improved yield whereas high pH (8.0) affected yield negatively. No significant affect on purity was seen with changing pH. Additives such as DTT, glycerol, and NaCl did not significantly affect the purification of this particular protein.

The screening results showed that the optimal buffer conditions for purifying GST-hippocalcin with highest yield and purity were: 10–20 mM sodium phosphate, 140–400 mM NaCl, pH 6.2–7.0 (Table 4). Results reflecting sample preparation showed in this case that both the commercial cell lysis kit and sonication can be used to lyse *E. coli* without significantly affecting the purification result.

SDS-PAGE of GST-hippocalcin purified on GST MultiTrap FF showed no significant difference in purity of the target protein resulting from the various buffer conditions (Fig 5).

96-well filter plate:	GST MultiTrap FF
Sample:	Unclarified *E. coli* BL21 lysate containing GST-tagged hippocalcin, M_r 45 000
Purification protocol:	According to GST MultiTrap Instructions, 28-4070-75
Sample preparation:	Lysis using CelLytic B Plus Kit and sonication were compared. Both methods were performed according to standard protocols.
Sample volume:	500 µl
Elution volume:	3 × 200 µl
Binding buffer:	Parameters varied and randomly tested: 10–20 mM PBS; 50–100 mM Tris-HCl; pH 6.2–8.0; 140–400 mM NaCl; 0–5 mM DTT; 0% to 5% glycerol and 0–2 mM glutathione.
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Elution method:	Centrifugation
Data evaluation:	MODDE software, UV-spectrometry (A_{280}), SDS-PAGE



Lanes
1. LMW markers
2. Start material
3. Sonication, 10 mM PBS, 140 mM NaCl, pH 7.4
4. CelLytic kit, 10 mM PBS, 140 mM NaCl, pH 7.4
5. CelLytic kit, 10 mM PBS, 400 mM NaCl, 2 mM glutathione, 5% glycerol, pH 8
6. Sonication, 20 mM PBS, 400 mM NaCl, 5% glycerol, pH 6.2
7. Sonication, 20 mM PBS, 400 mM NaCl, 2 mM glutathione, pH 8
8. Sonication, 50 mM Tris-HCl, 400 mM NaCl, 5% glycerol, pH 6.2
9. Sonication, 50 mM Tris-HCl, pH 8
10. Sonication, 50 mM Tris-HCl, 140 mM NaCl, 2 mM glutathione, 5 mM DTT, 5% glycerol, pH 8
11. Sonication, 100 mM Tris-HCl, 140 mM NaCl, 5 mM DTT, pH 6.2
12. Sonication, 100 mM Tris-HCl, 270 mM NaCl, 1 mM glutathione, 2.5 mM DTT, 2.5% glycerol, pH 7.4

Fig 5. SDS-PAGE (reducing conditions, ExcelGel SDS Gradient 8–18; Coomassie™ staining) of collected fractions of eluted GST-hippocalcin from some of the GST MultiTrap FF filter plate wells. See Table 4 for additional details on yield and purity.

Ordering information

Product	Quantity	Code no.
GST MultiTrap FF	4 × 96-well filter plates	28-4055-01
GST MultiTrap 4B	4 × 96-well filter plates	28-4055-00

Accessories	Quantity	Code no.
Collection Plate, 96-well plate 500 µl, V-shaped bottom	5 pack	28-4039-43

Related products	Quantity	Code no.
GSTrap FF*	Various	Various
GSTPrep FF 16/10	1 × 20 ml	17-5234-01
Glutathione Sepharose 4 Fast Flow†	Various	Various
GSTrap 4B‡	Various	Various

* Available in a range of standard pack sizes; bulk pack sizes of 100 × 1 ml and 100 × 5 ml are available by special order. Please contact your local sales office or consult the web site for more information.

† Available in 25, 100, and 500 ml pack sizes.

‡ Available in a range of standard pack sizes; bulk pack sizes of 100 × 1 ml and 100 × 5 ml are available by special order.

§ Available in 10, 100, and 300 ml pack sizes.

¶ All pGEX vectors include *E. coli* BL21 cells.

Related products (cont.)	Quantity	Code no.
Glutathione Sepharose 4B§	Various	Various
GST MicroSpin™ Purification Module	50 columns	27-4750-03
Glutathione S-transferase gene fusion vectors (pGEX vectors)¶	Various	Various
GST 96-Well Detection Module	5 plates	27-4592-01
Anti-GST Antibody (50 detections)	0.5 ml	27-4577-01
E. coli BL21¶	1 vial	27-1542-01

Literature	Quantity	Code no.
GST Gene Fusion System Handbook	1	18-1157-58
Recombinant Protein Handbook	1	18-1142-75
Affinity Chromatography Handbook	1	18-1022-29
Affinity Chromatography Columns and Media Product Profile	1	18-1121-86

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A license for commercial use of GST gene fusion vectors must be obtained from Chemicon International, Incorporated, 28820 Single Oak Drive, Temecula, California 92590, USA.

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