

Selection guide

# Solutions for protein preparation and detection of GST-tagged proteins



# Glutathione Sepharose

A variety of affinity chromatography products are available from Cytiva. For the purification of GST-tagged recombinant proteins, three different chromatography media are available — Glutathione Sepharose™ High Performance (HP), Glutathione Sepharose 4 Fast Flow (FF) and Glutathione Sepharose 4B. Glutathione Sepharose media are available in several formats, ranging from 96-well filter plates, spin columns, gravity flow columns to prepacked HiTrap™ and HiPrep™ columns and lab packs (media packs in sizes from 25 to 500 ml). The media vary in their performance parameters, and the different formats provide options for scale and convenience.

Glutathione Sepharose media offer these benefits:

- Mild, nondenaturing conditions preserves protein antigenicity and function
- High purity in a single step

The binding of a GST-tagged protein to the ligand is reversible, and the protein can be eluted under mild, nondenaturing conditions by the addition of reduced glutathione to the elution buffer. The technique thus provides a purification process that preserves protein antigenicity and function.

## Glutathione Sepharose 4B

### Protein preps by batch/column

Glutathione Sepharose 4B provides high binding capacity, approx. 25 mg/ml medium, and is suitable for small-scale purification as well as batch and gravity flow operations. The medium is also available prepacked in 96-well plates for screening purposes and in convenient column formats.

### GSTrap 4B prepacked columns for easy purification

GSTrap™ 4B 1-ml and 5-ml columns are prepacked with Glutathione Sepharose 4B for easy purification of GST-tagged proteins at laboratory scale. The columns can be used with a syringe, a pump, or a chromatography system such as ÄKTA design. In addition, multiple GSTrap 4B columns can be easily connected in series for increased purification capacity.

### GST MultiTrap 4B 96-well filter plates for high-throughput screening

GST MultiTrap™ 4B are prepacked 96-well filter plates for convenient high-throughput parallel screening of GST-tagged proteins. Each well is packed with Glutathione Sepharose 4B. Unclassified cell lysates can be loaded directly into the wells. Consistent well-to-well and plate-to-plate performance ensures high reproducibility. GST MultiTrap 4B filter plates simplify and shorten the time for expression screening, thereby helping to minimize the degradation of target proteins. Operation is possible with robotic systems or manually by centrifugation or vacuum. For scale-up, use GSTrap 4B columns.

### GST SpinTrap for protein minipreps

GST SpinTrap™ columns are prepacked with Glutathione Sepharose 4B and are designed for small-scale purification of GST-tagged proteins from clarified cell lysates. The columns are also suitable for expression screening. GST SpinTrap columns are used with a standard microcentrifuge.

### GST GraviTrap columns

GST GraviTrap™ provides convenient, disposable columns prepacked with 2 ml of Glutathione Sepharose 4B. Each package contains 10 prepacked columns designed for gravity purification. GST GraviTrap columns are delivered in a package that converts conveniently into a column stand (Workmate). The plastic tray in the product package can be used to collect liquid waste.

### GST Bulk Kit

GST Bulk Kit contains a 10 ml bulk pack of Glutathione Sepharose 4B and five disposable columns together with ITPG and GST Buffer Kit. With this kit, GST-tagged proteins can be purified using either column chromatography or a batch method.

## Glutathione Sepharose 4 Fast Flow

### Protein preps by batch/column and scale-up

Glutathione Sepharose 4 Fast Flow provides good protein binding capacity. The good flow properties of the 90 µm highly cross-linked agarose matrix make it an excellent choice for protein preps (> 1 mg) by batch or column purification and scale-up. Glutathione Sepharose 4 Fast Flow is available in lab packs, convenient column formats for scale-up, and 96-well filter plates.

### GSTrap FF and GSTPrep FF 16/10 prepacked columns for fast and easy scale-up

GSTrap FF 1-ml and 5-ml columns are prepacked with Glutathione Sepharose 4 Fast Flow and enable purifications of GST-tagged proteins to be easily scaled up. The columns can be used with a syringe, a pump, or a chromatography system such as ÄKTAdesign. The high flow rates made possible by the Sepharose 4 Fast Flow matrix make GSTrap FF columns an excellent choice for scale-up. In addition, multiple GSTrap FF columns can be connected in series for increased purification capacity. Prepacked GSTPrep™ FF 16/10 is a 20-ml column that provides additional capacity.

### GST MultiTrap FF 96-well filter plates for high-throughput screening

GST MultiTrap FF are prepacked 96-well filter plates for convenient high-throughput parallel screening of GST-tagged proteins. Each well is packed with Glutathione Sepharose 4 Fast Flow. Unclassified cell lysates can be loaded directly into the wells. Consistent well-to-well and plate-to-plate performance ensures high reproducibility. GST MultiTrap FF filter plates simplify and shorten the time for expression screening, thereby helping to minimize the degradation of target proteins. Operation is possible with robotic systems or manually by centrifugation or vacuum. For scale-up, use GSTrap FF and GSTPrep FF 16/10 columns.

## Glutathione Sepharose High Performance

### For high-resolution purification

Glutathione Sepharose High Performance is a highly cross-linked 34 µm beaded agarose and therefore an excellent choice for high-resolution purification. Glutathione Sepharose High Performance ensures the lowest sample dilution and best high-resolution separation. Glutathione Sepharose High Performance is available in lab packs and convenient, prepacked, GSTrap HP 1-ml and 5-ml columns.

### GSTrap HP columns for convenient and reproducible purification

GSTrap HP 1-ml and 5-ml columns are prepacked with Glutathione Sepharose High Performance for reliable, high-resolution purification of GST-tagged proteins at laboratory scale. The columns can be used with a syringe, a pump, or a chromatography system such as ÄKTAdesign.

# GST Gene Fusion System

Glutathione S-transferase (GST) Gene Fusion System is an integrated system for the expression, purification, and detection of GST-tagged proteins produced in *E. coli*.

## pGEX vectors

GST-tagged proteins are constructed by inserting a gene or gene fragment into the multiple cloning site of one of the pGEX vectors. The vectors provide all three translational reading frames beginning with the EcoRI restriction site. The pGEX vectors are designed for inducible, high-level intracellular expression of genes or gene fragments. Expression in *E. coli* yields tagged proteins with the GST moiety at the amino terminus and the protein of interest at the carboxyl terminus. Thirteen pGEX vectors are available (see *Ordering information*); all of them have a tac promoter for chemically inducible, high-level expression and an internal lac1q gene for use in any *E. coli* host.

## Detection of GST

### Anti-GST Antibody

Anti-GST Antibody is a polyclonal antibody purified from the sera of goats for highly sensitive and specific detection of recombinant GST-tagged proteins. The strength of a polyclonal antibody is that it can recognize different GST epitopes, so that GST-tagged proteins are detected even if some binding sites are masked due to protein folding. Anti-GST Antibody is supplied unconjugated for use with any enzyme-conjugated anti-goat antibody, and is recommended for use in Western blots and dot blots.

**GST 96-Well Detection Module**

GST 96-Well Detection Module permits rapid, sensitive determination of GST fusion proteins in a variety of samples. Clarified lysates or intermediate purification fractions can be applied directly into the wells of GST 96-Well Detection Plates. GST-tagged proteins are captured on Anti-GST Antibody immobilized on the walls of each well. Captured GST-tagged proteins are detected with HRP/Anti-GST Conjugate provided in the module. Standard curves for quantitation of GST-tagged proteins can be made with recombinant GST (rGST).

**GST Detection Module**

GST Detection Module enables sensitive detection of GST-tagged proteins and contains components for detection using either a biochemical assay where glutathione and 1-chloro-2-4-dinitrobenzene (CDNB) serve as substrates for GST to yield a yellow product detectable at 340 nm, or an immunoassay.

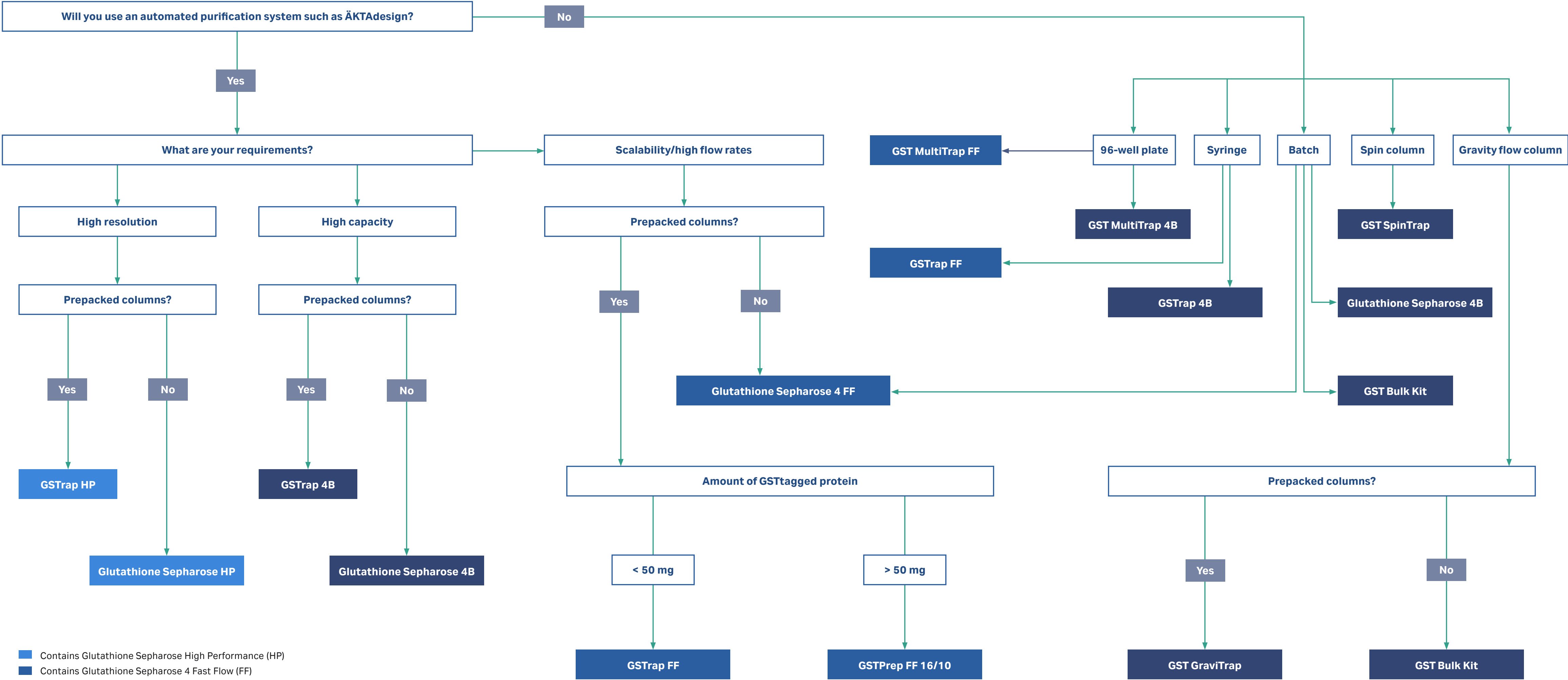
**Removal of GST tag by enzymatic cleavage**

Removal of the GST tag can be performed before functional or structural studies of the target protein. The amount of protease, temperature, and length of incubation required for complete digestion varies according to the nature of the target protein. Tagged proteins containing a PreScission™ Protease, Thrombin, or Factor Xa recognition site can be cleaved either while bound to Glutathione Sepharose chromatography media or in solution after elution. When the GST protein is bound to the column, cleavage releases the target protein, which is eluted with binding buffer while the GST moiety remains bound to the medium. On-column cleavage is generally recommended as the method of choice since many potential contaminants can be washed out and the target protein elutes with a higher level of purity. Cleavage after elution is suggested if optimization of cleavage conditions is necessary.

| Vector                          | Cleavage enzyme      |
|---------------------------------|----------------------|
| pGEX-6P-1, pGEX-6P-2, pGEX-6P-3 | PreScission Protease |
| pGEX-4T-1, pGEX-4T-2, pGEX-4T-3 | Thrombin             |
| pGEX-5X-1, pGEX-5X-2, pGEX-5X-3 | Factor Xa            |
| pGEX-2TK                        | Thrombin             |

For more information, see *GST Gene Fusion System handbook*, Code no. 18-1157-58 and the *Glutathione S-Transeferase (GST) Gene Fusion System Data file*, Code no. 28-9622-84 AA

# Selection Guide – Glutathione Sepharose products



■ Contains Glutathione Sepharose High Performance (HP)  
■ Contains Glutathione Sepharose 4 Fast Flow (FF)  
■ Contains Glutathione Sepharose 4B

# Products for protein purification

| Product                                | Product code no. | Pack size                | Approx. protein binding capacity* | High-throughput screening | Minipreps | Batch/ gravity flow | Syringe compatible | ÄKTA design system compatible | Data file code no. |
|----------------------------------------|------------------|--------------------------|-----------------------------------|---------------------------|-----------|---------------------|--------------------|-------------------------------|--------------------|
| Glutathione Sepharose High Performance | 17-5279-01       | 25 ml                    | 7 mg rGST/ml                      | •                         |           |                     |                    | •                             | 28-9941-47         |
|                                        | 17-5279-02       | 100 ml                   |                                   | •                         |           |                     |                    | •                             |                    |
| GSTrap HP                              | 17-5281-01       | 5 × 1 ml                 | 7 mg rGST/column                  |                           |           |                     |                    | •                             | 28-9941-47         |
|                                        | 17-5282-01       | 1 × 5 ml                 | 35 mg rGST/column                 |                           |           |                     |                    | •                             | 28-9941-47         |
|                                        | 17-5282-02       | 5 × 5 ml                 |                                   |                           |           |                     |                    | •                             |                    |
| Glutathione Sepharose 4 Fast Flow      | 17-5132-01       | 25 ml                    | 10 mg rGST/ml                     | •                         | •         | •                   |                    | •                             | 28-9941-47         |
|                                        | 17-5132-02       | 100 ml                   |                                   | •                         | •         | •                   |                    | •                             |                    |
|                                        | 17-5132-03       | 500 ml                   |                                   | •                         | •         | •                   |                    | •                             |                    |
| GSTrap FF                              | 17-5130-02       | 2 × 1 ml                 | 10 mg rGST/column                 |                           |           |                     | •                  | •                             | 28-9941-47         |
|                                        | 17-5130-01       | 5 × 1 ml                 |                                   |                           |           |                     | •                  | •                             |                    |
|                                        | 17-5131-01       | 1 × 5 ml                 | 50 mg rGST/column                 |                           |           |                     | •                  | •                             | 28-9941-47         |
|                                        | 17-5131-02       | 5 × 5 ml                 |                                   |                           |           |                     | •                  | •                             |                    |
| GSTPrep FF 16/10                       | 28-9365-50       | 1 × 20 ml                | 200 mg rGST/column                |                           |           |                     |                    | •                             | 28-9941-47         |
| GST MultiTrap FF                       | 28-4055-01       | 4 × 96-well filter plate | 500 µg rGST/well                  | •                         |           |                     |                    |                               | 28-4081-57         |
| Glutathione Sepharose 4B               | 17-0756-01       | 10 ml                    | 25 mg horse liver GST/ml          | •                         | •         | •                   |                    |                               | 28-9941-47         |
|                                        | 17-0756-04       | 300 ml                   |                                   | •                         | •         | •                   |                    |                               |                    |
|                                        | 17-0756-05       | 100 ml                   |                                   | •                         | •         | •                   |                    |                               |                    |
| GSTrap 4B                              | 28-4017-45       | 5 × 1 ml                 | 25 mg horse liver GST/column      |                           |           |                     | •                  | •                             | 28-9941-47         |
|                                        | 28-4017-47       | 1 × 5 ml                 | 125 mg horse liver GST/column     |                           |           |                     | •                  | •                             | 28-9941-47         |
|                                        | 28-4017-48       | 5 × 5 ml                 |                                   |                           |           |                     | •                  | •                             |                    |
| GST GraviTrap                          | 28-9523-60       | 10 × 2 ml                | 50 mg horse liver GST/column      |                           |           | •                   |                    |                               | 28-9622-84         |
| GST Bulk Kit                           | 27-4570-01       | 1 kit                    | 25 mg/ml medium                   |                           |           | •                   |                    |                               | 28-9622-84         |
| GST SpinTrap                           | 28-9523-59       | 50 × 50 µl               | 500 µg horse liver GST/column     |                           | •         |                     |                    |                               | 28-9622-84         |
| GST MultiTrap 4B                       | 28-4055-00       | 4 × 96-well filter plate | 500 µg horse liver GST/well       | •                         |           |                     |                    |                               | 28-4081-57         |

\* The binding of GST-tagged proteins depends on size, conformation, and concentration of the protein in the sample loaded. Binding of GST to glutathione is also flow dependent, and lower flow rates often increase the binding capacity. This is important during sample loading. Protein characteristics, pH, and temperature may also affect the binding capacity.

## Products for protein expression and detection

| Product             | Quantity | Code no.   |
|---------------------|----------|------------|
| <b>pGEX vectors</b> |          |            |
| pGEX-4T-1           | 25 µg    | 28-9545-49 |
| pGEX-4T-2           | 25 µg    | 28-9545-50 |
| pGEX-4T-3           | 25 µg    | 28-9545-52 |
| pGEX-5X-1           | 25 µg    | 28-9545-53 |
| pGEX-5X-2           | 25 µg    | 28-9545-54 |
| pGEX-5X-3           | 25 µg    | 28-9545-55 |
| pGEX-2TK            | 25 µg    | 28-9546-46 |
| pGEX-6P-1           | 25 µg    | 28-9546-48 |
| pGEX-6P-2           | 25 µg    | 28-9546-50 |
| pGEX-6P-3           | 25 µg    | 28-9546-51 |
| pGEX-2T             | 25 µg    | 28-9546-53 |
| pGEX-3X             | 25 µg    | 28-9546-54 |
| pGEX -1λT EcoRI/BAP | 5 µg     | 28-9546-56 |

| Product                                | Quantity              | Code no.   |
|----------------------------------------|-----------------------|------------|
| <b>Detection</b>                       |                       |            |
| Anti-GST Antibody                      | 0.5 ml, 50 detections | 27-4577-01 |
| GST Detection Module                   | 50 detections         | 27-4590-01 |
| GST 96-Well Detection Module           | 5 plates              | 27-4592-01 |
| Anti-GST HRP Conjugate                 | 75 µl                 | RPN1236    |
| ECL GST Western Blotting Detection Kit | 1 kit                 | RPN1237    |
| <b>Cleavage</b>                        |                       |            |
| Thrombin                               | 500 units             | 27-0846-01 |
| Factor Xa                              | 400 units             | 27-0849-01 |
| PreScission Protease                   | 500 units             | 27-0843-01 |

# Handbooks from Cytiva

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