

**Miltenyi Biotec GmbH**  
Friedrich-Ebert-Str. 68  
51429 Bergisch Gladbach, Germany  
Phone: +49 2204 83060  
Fax: +49 2204 85197  
macs@miltenyibiotec.de

**Miltenyi Biotec Inc.**  
12740 Earhart Avenue  
Auburn CA 95602, USA  
Phone: 800 FOR MACS, +1 530 888 8871  
Fax: +1 530 888 8925  
macs@miltenyibiotec.com

**Miltenyi Biotec Pty. Ltd. (Australia)**  
Phone: +61 02 8877 7400  
macs@miltenyibiotec.com.au

**Miltenyi Biotec B. V. (Benelux)**  
macs@miltenyibiotec.nl  
Customer service, Netherlands  
Phone 0800-4020120  
Customer service, Belgium  
Phone 0800 94016  
Customer service, Luxemburg  
Phone 800 24971

**Miltenyi Biotec Shanghai Office**  
Phone: +86 021 62351005  
miltenyibiotec@china.com

**Miltenyi Biotec (France)**  
Phone: +33 01 56 98 16 16  
macs@miltenyibiotec.fr

**Miltenyi Biotec S.r.l. (Italy)**  
Phone: +39 051 64 60 411  
macs@miltenyibiotec.it

**Miltenyi Biotec K.K. (Japan)**  
Phone: +81 3 56 46 8910  
macs@miltenyibiotec.jp

**Miltenyi Biotec Asia Pacific Pte. Ltd. (Singapore)**  
Phone: +65 6238 8183  
macs@miltenyibiotec.com.sg

**Miltenyi Biotec S.L. (Spain)**  
Phone: +34 91 512 12 90  
macs@miltenyibiotec.es

**Miltenyi Biotec Ltd. (UK)**  
Phone: +44 01483 799 800  
macs@miltenyibiotec.co.uk

For further information refer to our website [www.miltenyibiotec.com](http://www.miltenyibiotec.com)

For technical questions, please contact your local distributor or our Technical Support Team in Germany:

E-mail [macsTec@miltenyibiotec.de](mailto:macsTec@miltenyibiotec.de)  
Phone +49 2204 8306-830.

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This MACS® product is for *in vitro* research use only and not for diagnostic or therapeutic procedures.

## ProCatch Glutathione Resin User Manual

Order no. 130-092-187  
130-092-186  
130-092-185

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### 1. Description

<b>Components</b>	ProCatch Glutathione Resin 10 mL (# 130-092-187) 25 mL (# 130-092-186) 100 mL (# 130-092-185)
<b>Intended use</b>	Purification of glutathione-binding proteins
<b>Product format</b>	ProCatch Glutathione Resin contains agarose supplied as 50% slurry in 20% Ethanol Storage Buffer (pH 7.0).
<b>Storage</b>	Store the resin as a slurry in 20% ethanol at 4 °C.

#### ProCatch Glutathione Resin characteristics

Capacity	up to 15 mg glutathione-binding protein/mL resin
Matrix	Sepharose™ CL-4B (GE Healthcare)
Bead size	45–165 µm
Max. lineare flow rate*	80 cm/h
Max. operating pressure	14 psi = 0.1 MPa
pH stability	3–14

\* 
$$\text{volumetric flow} \left( \frac{\text{mL}}{\text{min}} \right) = \frac{\text{volumetric flow} \left( \frac{\text{cm}^3}{\text{h}} \right) \times \text{column cross-sectional area} \left( \text{cm}^2 \right)}{60 \left( \frac{\text{min}}{\text{h}} \right)}$$

The cover photo shows a replica of the DNA model built in 1953 by James D. Watson and Francis Crick at the Cavendish Laboratory in Cambridge. This model is located at Heureka, the Finnish Science Centre. Photography by Alexander Budde; © Miltenyi Biotec GmbH, Germany. Detailed information on the history of the Watson-Crick model can be found in: de Chadarevian, S. (2003) Relics, replicas and commemorations. Endeavour 27: 75–79.

### 1.1 Background and product applications

The major goal in protein purification is to reach high protein purities quickly with a method that can easily be applied to diverse proteins. Purifying proteins with a combination of “classical” methods such as ion-exchange chromatography and gel filtration is problematic as each protein will require development of a customized purification protocol.

In contrast, the use of specific protein-protein or protein-substrate interactions allows a one-step affinity purification independent of the protein itself. In the case of affinity chromatography using glutathione-based resins, a highly selective purification of proteins containing glutathione-binding sequences such as glutathione S-transferase (GST), glutathione peroxidase and glyoxalase I is possible.<sup>1,2</sup> ProCatch Glutathione Resin is a glutathione-agarose for high-affinity purification of glutathione-binding proteins. Using ProCatch Glutathione Resin, the glutathione-binding protein of interest can be isolated rapidly and effectively from the cell extract under mild, non-denaturing conditions.

#### ProCatch Glutathione Resin

ProCatch Glutathione Resin is a high performance glutathione agarose for the fast and selective purification of glutathione-binding proteins by affinity chromatography from cell lysates.<sup>1,2</sup> ProCatch Glutathione Resin has optimized structural characteristics for efficient purification of glutathione-binding proteins and preserves the functional integrity of the native protein. The binding capacity is up to 15 mg glutathione-S-transferase per mL resin.

After target protein binding, non-specific proteins are washed from the resin. A subsequent competitive elution of the target protein is achieved using reduced glutathione.

ProCatch Glutathione Resin shows high stability to a wide range of additives. It is compatible with a variety of compounds in wash and elution buffers (see 7. Appendix). ProCatch Glutathione Resin can be reused (see 3.6); although, to avoid cross-contamination this is only recommended with identical samples.

#### Product applications

ProCatch Glutathione Resin was developed for large-scale, fast purification and high recovery of glutathione-binding proteins. ProCatch Glutathione Resin offers exceptional performance and is compatible with all prokaryotic and also eukaryotic expression systems under non-denaturing conditions. It was designed for multiple preparation formats such as batch - gravity flow purification, gravity flow purification and low pressure column purification.

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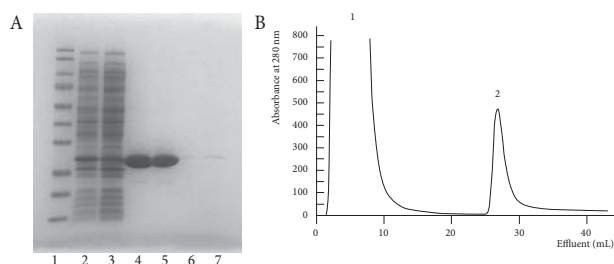


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**Figure 1: Purification of a 30 kDa glutathione-binding protein with ProCatch Glutathione Resin.**

A: Marker (lane 1), lysate from cell extract (lane 2), flow-through (lane 3), first eluate (lane 4), second eluate (lane 5), third eluate (lane 6) and eluate after boiling the resin (lane 7) were loaded onto a Coomassie-stained SDS gel.

B: Low pressure column purification protocol. Peak 1 defines the non-bound protein fraction (flow-through). Peak 2 corresponds to the eluted glutathione-binding protein. Proteins leaving the column were monitored by measuring absorbance at 280 nm.

### 1.2 Reagent and instrument requirements

#### Required buffers

- Prokaryotic Lysis / Binding Buffer:
  - 1× Phosphate buffered saline (PBS): 140 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8 mM KH<sub>2</sub>PO<sub>4</sub>, 2.4 mM KCl (pH 7.5)
- Eukaryotic Lysis / Binding Buffer:
  - 1% Triton<sup>®</sup> X-100, PBS
- Wash Buffer:
  - 1× PBS
- Elution Buffer (prepare fresh prior to use):
  - 10 mM reduced glutathione in 50 mM Tris-HCl (pH 8.0)
- Resin Regeneration Buffer 1:
  - 0.1 M Tris-HCl, 0.5 M NaCl (pH 8.5)
- Resin Regeneration Buffer 2:
  - 0.1 M Sodium acetate, 0.5 M NaCl (pH 4.5)
- Resin Regeneration Buffer 3:
  - 1× PBS
- Ethanol Storage Solution:
  - Ethanol diluted to 20% in distilled water

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**Required reagents**

- (Optional) Nuclease, e.g. Benzonase® Nuclease (Novagen, # 71205), or DNase I

**Required disposables**

- Gravity flow column with end-caps (for gravity flow purification)
- Chromatography column (for low pressure column purification)
- Polypropylene tubes

**Required instruments**

- Centrifuge (precooled to 4 °C)
- Chromatography system (for automated low pressure purification method)
- Ultrasonic homogenizer (sonicator)
- MACSmix™ Tube Rotator (# 130-090-753)

**1.3 Related products**

For highly pure small scale isolation of GST-tagged proteins, the μMACS GST Protein Isolation Kit (# 130-091-370) is recommended. This kit allows the magnetic affinity purification based on monoclonal antibodies.

**2. Purification strategy**

▲ To prevent protein degradation of native proteins, perform all work, especially the lysis, on ice (4–8 °C).

▲ Proteinase inhibitors can be added to the Lysis Buffer. For details, refer to the appendix where appropriate inhibitors and effective concentrations are listed.

▲ ProCatch Glutathione Resin is recommended for gravity purification or batch-gravity purification. For the batch-gravity purification use low speed centrifugation (700×g) to pellet the resin

▲ The resin is provided as 50% glutathione sepharose slurry. To obtain the desired bed volume, use twice the volume (e.g. 2 mL of resuspended 50% glutathione sepharose will result in a bed volume of 1 mL).

▲ Due to the relatively slow binding kinetics between glutathione and glutathione-binding protein, the flow rate is one important parameter which affects the quality and quantity of the purification. To achieve a maximum binding capacity of the ProCatch Glutathione Resin, it is very important to keep the flow rate low when the sample is applied.

▲ Do **not use** strong denaturants like urea or guanidinium in the purification buffers. These denature the glutathione-binding protein.

**Protein purification using ProCatch Glutathione Resin**

ProCatch Glutathione Resin is optimized for protein purification from *E. coli* and insect cell cultures. It can additionally be used for large scale protein isolations from other eukaryotic cells.

The following table gives an overview of possible protein purification methods and applications.

**Table 1: Protein purification with ProCatch Glutathione Resin**

Method	Recommended use
Batch-gravity flow (see 3.2)	Purification with more than 1 mL of resin bed volume, i. e. more than 2 mL of resin slurry
Gravity flow (see 3.3)	Rapid purification with up to 1 mL of resin bed volume, i. e. up to 2 mL of resin slurry
Automated low pressure (see 3.4)	Flow-regulated column purification in chromatography system
Test scale (see 3.5)	Test of expression level of target protein

**Lysis**

The lysis is one of the most crucial steps during protein purification. For special applications the Lysis Buffer has to be adapted. Factors such as ionic strength, pH, the concentration and type of detergent, the presence of divalent cations, co-factors, and stabilizing ligands influence the effectiveness of the Lysis Buffer. Generally, the Lysis Buffer should not contain SDS as it denatures proteins.

▲ Do **not use** lysozyme for lysis of the cells because it may interfere with the functionality of the target protein.

▲ Harvested eukaryotic cells should be lysed in an appropriate buffer containing a mild detergent (see 1.2).

▲ When sonicating cells for lysis, check viscosity of the sample. Genomic DNA in the solution, which is not completely sheared, increases sample viscosity and can affect the recovery of the protein. Decrease the sample's viscosity by degradation of DNA and RNA with nuclease (usage as recommended by the manufacturer; be aware that nucleases require Mg<sup>2+</sup>). This should improve the yield of protein. Alternatively, dilute the sample five-fold with Binding/Wash Buffer before applying it to the resin.

**Elution**

Generally, an elution buffer containing 10 mM of reduced glutathione will elute the target protein efficiently. However, higher concentrations (20–50 mM) may result in a better recovery of some glutathione-binding proteins such as GST. If the glutathione concentration is above 15 mM, the buffer concentration should be raised and if necessary the pH adjusted to between 8 and 9.

Additional elution steps should be performed during the purification process if a high amount of glutathione-binding protein remains on the column.

The flow-through and eluates from the column can be monitored for glutathione-binding proteins by using SDS-PAGE and Western blot analysis to examine which fractions contain the majority of target protein. To determine the yield of purified protein, use a Bradford protein assay<sup>3</sup> as the glutathione will not interfere with this method. UV measurement at 280 nm cannot be performed because the glutathione masks the absorbance of the eluted protein.

### 3. Protocols

#### 3.1 Sample preparation

##### 3.1.1 Lysate from prokaryotic cells

The expression rate of proteins is highly variable and must be determined empirically, e.g. by SDS-PAGE. One mL of ProCatch Glutathione Resin bed volume is equal to 2 mL of the 50% slurry used and can bind up to 15 mg glutathione-binding protein. The binding capacity of the resin depends on the specific protein. Calculate the bed volume of ProCatch Glutathione Resin for the amount of cells used for purification and for the anticipated yield of protein.

1. Centrifuge the cell culture at 3,000–5,000g for 15 min at 4 °C to harvest the cells. Decant the supernatant.
2. Resuspend the cell pellet by vortexing in 2 mL pre-cooled Prokaryotic Lysis/Binding Buffer (4 °C) per 25 mL culture.

(Optional) Add protease inhibitors such as PSMF and leupeptin (see 7.3).

3.
  - For sample volume up to 200 mL: sonicate the sample 3×10 sec, interrupted by a 30-sec pause on ice between each sonication step.
  - For sample volume ≥ 200 mL: sonicate the sample 3×30 sec, interrupted by a 2-min pause on ice between each sonication step.

▲ **Note:** Excessive sonication can destroy protein functionality.

4. (Optional) If the viscosity of the lysate is high, add nuclease, e.g. 1 µL (25 units) Benzonase Nuclease per mL of buffer and incubate for 15 min on ice.
5. Centrifuge the lysate at 10,000–15,000×g for 30 min at 4 °C to pellet the cell debris.
6. Transfer the supernatant carefully to a clean tube. Do not disturb the pellet. The clarified sample contains the protein which can be analyzed by taking a small part (1–10 µL) for SDS-PAGE analysis.
7. Continue with protein purification (see 3.2–3.5).

##### 3.1.2 Lysate from eukaryotic cells (native)

The expression rate of proteins is highly variable and must be determined empirically, e.g. by SDS-PAGE. The binding capacity of the resin depends on the specific protein. One mL of ProCatch Glutathione Resin bed volume is equal to 2 mL of the 50% slurry used and can bind up to 15 mg glutathione-binding protein. Calculate the bed volume of ProCatch Glutathione Resin for the amount of cells used for purification and for the anticipated yield of protein.

1. Transfer the cells to a centrifugation tube and centrifuge for 5 min at 300×g at 4 °C.
2. Remove supernatant. Place the tube containing the cell pellet on ice and add 1 mL of Eukaryotic Lysis/Binding Buffer per 10<sup>7</sup> cells. Mix well by vortexing.  
(Optional) Add protease inhibitors such as PSMF and leupeptin (see 7.2).

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3. Incubate on ice for 15 min with occasional vortexing.
4. Centrifuge at 10,000–15,000×g for 30 min at 4 °C to sediment the cell debris.
5. Transfer the supernatant to a fresh tube and proceed with the protein purification.

#### 3.2 Batch - gravity flow column protein purification

This protocol is for purification with more than 1 mL of resin bed volume. One bed volume of ProCatch Glutathione Resin (e.g. 1 mL) is obtained by using double the volume (e.g. 2 mL) of resuspended resin.

##### 3.2.1 Preparation of the ProCatch Glutathione Resin

1. Resuspend the ProCatch Glutathione Resin thoroughly.
2. Transfer the required amount of resin suspension to a sterile tube that has enough volume for 10–20 times the resin bed volume.
3. Pellet the resin by centrifugation at 700×g for 2 min.
4. Carefully discard the supernatant.
5. Pre-equilibrate the resin by adding 10 volumes of Wash Buffer and mix briefly.
6. Pellet the resin by centrifugation at 700×g for 2 min and discard the supernatant.
7. Repeat steps 5 and 6.

##### 3.2.2 Batch-gravity flow column glutathione-binding protein purification

1. The cleared cell lysate can now be added to the ProCatch Glutathione Resin.
2. Put the sample into the MACSmix Tube Rotator and rotate for 20 min to bind the target protein to the resin.
3. Pellet the resin by centrifugation at 700×g for 5 min.
4. Carefully remove as much supernatant as possible. Do not touch the pellet.
5. Wash the resin by adding 10–20 volumes of Wash Buffer (pH 7.5) to resuspend the pellet. Rotate for 10 min in the MACSmix Tube Rotator.
6. Pellet the resin by centrifugation at 700×g for 5 min.
7. Discard the supernatant.
8. Repeat steps 5–7.
9. Add one volume Wash Buffer to the resin and resuspend by vortexing.
10. Transfer the resin to a 2 mL gravity-flow column (with end-cap) and remove air bubbles. Let the resin settle down.
11. Allow the buffer to drain by removing the end-cap of the column until the buffer surface reaches the top of the resin bed.

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- Add 5 bed volumes of Elution Buffer to the column to elute the glutathione-binding protein. Collect the eluate in 0.5–1 mL fractions and store on ice.
- To determine which fractions contain the highest amount of the target protein, a Bradford protein assay<sup>3</sup> and SDS-PAGE analysis can be used. In most cases, the majority of the protein will be in the first two bed volumes.

### 3.3 Gravity flow column protein purification

This protocol is for purification of up to 1 mL of resin bed volume. One bed volume of ProCatch Glutathione

#### 3.3.1 Preparation of ProCatch Glutathione Resin in the column

- Resuspend the ProCatch Glutathione Resin thoroughly
- Transfer the appropriate volume of the resuspended resin to the disposable column sealed with an end-cap. One bed volume of ProCatch Resin (e.g. 1 mL) is obtained by using double the volume (e.g. 2 mL) of resuspended resin. Remove air bubbles in the resin.
- Let the resin settle down completely in the column and let the liquid pass through.
- Apply 10 bed volumes of Wash Buffer to equilibrate the column. Avoid air bubbles and do not disturb the resin.

### 3.3.2 Gravity flow column protein purification

- Apply your sample to the column.
- Wash the column with 10 bed volumes of Wash Buffer.
- Add 5 bed volumes of Elution Buffer to the column to elute the glutathione-binding protein. Collect the eluate in 0.5–1 mL fractions and store on ice.
- To determine, which fractions contain the highest amount of the target protein, a Bradford protein assay<sup>3</sup> and SDS-PAGE analysis can be used. In most cases, the majority of the protein will be in the first two bed volumes.

### 3.4 Automated low pressure column protein purification

This protocol is for automated flow-regulated column purification in chromatography systems.

▲ Use a flow rate of 75 cm/h for column packing and a flow rate of 30–60 cm/h for all other steps. A flow rate >80 cm/h will damage the resin.

▲ It is recommended to load the sample at a flow rate of 30–60 cm/h. If the protein does not bind, reduce the flow rate. Do not change the flow rate between equilibration and sample loading.

▲ If the target protein is unstable at room temperature, perform the chromatography at 4 °C.

Assemble the column according to the manufacturer's instructions.



- Resuspend the ProCatch Glutathione Resin and pour the slurry into the column. Avoid air bubbles in the column.
- Let the resin settle down. The process can be accelerated by applying a pump attached to the column output which allows the buffer to flow through. For packing, use a linear flow rate of 75 cm/h. Do not allow the resin to dry out.
- Adjust the top adaptor to the column and connect it to the chromatography system according to manufacturer's instructions.
- Equilibrate the column with 5 column volumes Wash Buffer. Check the eluate at 280 nm. Wash with Wash Buffer until the absorbance reaches the baseline.
  - ▲ Note: Do not exceed a flow rate of 60 cm/h for equilibration.
- Centrifuge the sample at  $\geq 15,000 \times g$  for 20–30 min at 4 °C and/or filter the sample through a 0.45  $\mu\text{m}$  filter and apply the pretreated sample to the column. Start collecting the fractions.
- Wash the column with Wash Buffer until the baseline (280 nm) is stable (approx. 5–10 column volumes).
- Elute the target protein by applying 5 column volumes of Elution Buffer. Usually the glutathione-binding protein elutes in the fractions containing the second and third column volumes. Collect the eluate in appropriate fraction volumes and store on ice.
- Check which fractions contain the majority of the target protein by Bradford protein assay<sup>3</sup> and SDS-PAGE analysis.

### 3.5 Test scale protein purification

This protocol is intended to:

- check for the presence of glutathione-binding proteins.
- examine the expression levels of the protein.

Please note that the test scale purification does not yield very pure protein.

▲ It is recommended to monitor the experimental procedure: Take a small sample (10–50  $\mu\text{L}$ ) after each critical step and analyse these samples by SDS-PAGE.

▲ This purification should be performed in a batch format with 50  $\mu\text{L}$  bed volume of ProCatch Glutathione Resin.

Prepare a cleared cell lysate from 1.5 mL bacterial cell culture or  $0.5-1 \times 10^7$  eukaryotic cells in 0.5 mL Prokaryotic or Eukaryotic Lysis/Binding Buffer according to the protocols in section 3.1.

#### 3.5.1 Preparation of the ProCatch Glutathione Resin

- Resuspend the ProCatch Glutathione Resin thoroughly.
- Transfer 100  $\mu\text{L}$  of the resuspended resin to a sterile 1.5 mL microcentrifuge tube.
- Pellet the resin by centrifugation at  $700 \times g$  for 2 min at 4 °C.
- Carefully discard the supernatant.



- Pre-equilibrate the resin by adding 20 bed volumes (1 mL) of cold (4 °C) Wash Buffer (pH 7.5) and mix briefly.
- Pellet the resin by centrifugation at 700×g for 2 min at 4 °C.
- Discard the supernatant.
- Repeat the steps 5–7.

### 3.5.2 Test scale protein purification

- Retain 50 µL of the cleared lysate from the previous small scale sample preparation for later analysis. Transfer the remaining lysate to the microcentrifuge tube containing 50 µL of the above prepared ProCatch Glutathione Resin
- Gently mix the sample at room temperature for 10 min using the MACSmix Tube Rotator.
- To pellet the resin with bound protein, centrifuge at 14,000×g for 1 min at 4 °C.
- Carefully remove the supernatant and save a 50 µL aliquot for analysis.
- Add 1 mL of Wash Buffer.
- Vortex briefly until the solution is resuspended.
- To pellet the resin, centrifuge at 14,000×g for 1 min at 4 °C.
- Discard the supernatant and retain a 50 µL aliquot for analysis.
- Repeat steps 5–8.

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- Add 50 µL of Elution Buffer to the pellet and vortex briefly to elute the bound glutathione-binding protein.
- Centrifuge briefly at 14,000×g.
- Carefully transfer the supernatant to a fresh tube. This fraction contains the glutathione-binding protein.
- Repeat steps 10–12.
- Analyze fractions of interest by SDS-PAGE.

### 3.6 Regeneration of ProCatch Glutathione Resin

ProCatch Glutathione Resin can be regenerated for reuse. To avoid cross-contamination it is only recommended with identical samples.

- Wash the resin-filled column by adding 10 resin bed volumes of Resin Regeneration Buffer 1.
- Wash the resin-filled column by adding 10 resin bed volumes of Resin Regeneration Buffer 2.
- Repeat steps 1 and 2 twice.
- Equilibrate the resin-filled column by adding 10 resin volumes of Resin Regeneration Buffer 3.
- If the column is not to be used directly, rinse the column with 10 bed volumes of 20% ethanol and store the sealed column in 20% ethanol solution at 4 °C.



Denatured, precipitated or non-specific resin-bound protein may negatively influence the proper function of the ProCatch Glutathione Resin. To remove contamination from the resin proceed as follows.

- Wash the column with 2 column volumes 6 M Guanidinium-Cl.
- Immediately, wash the column with 5 column volumes of Resin Regeneration Buffer 3.

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### 4. Tips & hints

**Detergents**—these are partially hydrophobic and partially hydrophilic and can solubilize membranes and membrane proteins. They can be used to increase protein solubility and to decrease aggregation. Non-ionic detergents such as Triton X-100 and Tween 20 tend to be more gentle in their actions than ionic detergents and are more suitable for protein-protein interaction studies.

**Salts**—increasing the salt concentration in the buffer will decrease ionic interactions between proteins in a cell lysate. Up to 500 mM NaCl may be helpful to reduce binding of non-specific proteins.

**pH**—increasing or decreasing the pH of the buffer will change the net charge of the proteins depending on their pI and therefore influence the extent of non-specific ionic interactions. For an effective protein binding, the Lysis / Binding Buffer should have a pH of 6.5–8.0.

**DTT and β-ME**—these reducing agents are often used to prevent loss of enzyme function via oxidation and to prevent protein aggregation due to malformed disulfide bridges during protein isolation.

**EDTA**—this chelation agent binds divalent cations and can be used to prevent the action of certain enzymes that require ions such as Mg<sup>2+</sup> or Ca<sup>2+</sup>. It can also prevent protein-protein interactions that are dependent on the presence of divalent cations.

**Phosphatase inhibitors**—when active kinases or phosphorylated proteins are to be isolated, we recommend the addition of 1 mM activated sodium orthovanadate (not compatible with DTT) and 1–10 mM sodium fluoride to inhibit phosphatase activity.



## 5. Troubleshooting

### Viscosity of the sample is too high

High concentration of nucleic acid in the lysate:

- continue sonication until the viscosity is reduced, add nuclease to 5 µg/mL and incubate on ice for 10–15 min.
- dilute the sample 1:5 with additional Lysis/ Binding Buffer.

### Glutathione-binding protein is found in the flow-through fraction

Increase binding of glutathione-binding proteins to the resin:

- before cell lysis, add 1–10 mM DTT.
- decrease the flow rate to improve binding.

Sonication is excessive:

- avoid over-sonication, otherwise the glutathione-binding protein could be denatured. Check lysate microscopically to monitor the cells.

Binding Buffers are incorrect:

- check pH and the composition of all buffers. The pH should be in a range of 6.5–8.0.
- equilibrate ProCatch Glutathione Resin before use.

### No glutathione-binding protein is found in any fraction

Sonication is insufficient:

- check cell disruption by measuring the release of nucleic acids at A260 nm. A solution containing 50 µg per mL of double stranded DNA has an absorbancy (optical density) of 1.0 at a wave length of 260 nm. Use longer sonication times.

Protein is encapsulated in inclusion bodies:

- use denaturants, e.g. 6 M guanidinium-Cl or 8 M urea, or use detergent, e.g. 1% Triton X-100 to solubilize the inclusion bodies.<sup>4</sup> Triton X-100 can be added to the recommended Lysis/ Binding Buffer whereas denaturants must be removed by dialysis or by desalting to allow refolding of the protein before purification.<sup>5,6</sup> Several refolding methods are available. Select one which best suits the application.<sup>6,7</sup>

### Glutathione-binding protein is not present in the eluate

Inadequate glutathione concentration:

- concentration of glutathione in Elution Buffer should be increased. If the glutathione concentration is above 15 mM, the buffer concentration should be checked and, if necessary, adjusted to a pH range within 8–9.

pH of Elution Buffer is too low:

- increase pH. Increasing the pH of the Elution Buffer to pH 9 may improve elution.

Flow rate is too high:

- decrease flow rate to improve elution.

Ionic strength is too low:

- add 0.1–0.2 M NaCl to the Elution Buffer to increase ionic strength. A possible precipitation of hydrophobic proteins under high salt conditions can be prevented by adding a non-ionic detergent, e.g. 0.1% Triton X-100.

Non-specific hydrophobic binding:

- reduce non-specific hydrophobic binding that may prevent elution of the target protein, add a non-ionic detergent, e.g. 0.1% Triton X-100.

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Contamination with proteases:

- add protease inhibitors such as 1 mM PMSF to Lysis/ Binding Buffer.
- use a protease-deficient host strain like *E.coli* B21.

Not enough Elution Buffer used:

- use larger volumes of Elution Buffer.

### Eluted protein contains contaminations

Not enough Wash Buffer used:

- use larger volumes of Wash Buffer.

Wrong column size:

- if the bed volume is too large, reduce the amount of ProCatch Glutathione Resin. For highest purity, all glutathione groups should be occupied with glutathione-binding proteins. Therefore, slightly overloading the resin with glutathione-binding proteins is advisable.

Protein sample applied to the column is too concentrated:

- make a 1:5 or 1:10 dilution with additional buffer and centrifuge.

Excessive non-specific interactions:

- add non-ionic detergent (e.g. up to 2% Triton X-100 or 2% Tween 20) to Wash Buffer.
- add salt (e.g. 0.1–0.2 M NaCl) to Wash Buffer.
- an additional purification step can be included (e.g. antibody affinity chromatography, ion exchange chromatography) to remove contaminants. Refer to literature for additional information about methods for separating glutathione-binding proteins from co-purifying proteins such

as chaperonins.<sup>8</sup> For example, the 70 kDa contaminant DnaK can be removed by pre-incubation of the bacterial cell lysate with ATP.<sup>9</sup>

### Column matrix is blocked

Cellular debris clogged the frit / filter:

- change the frit / filter.
- completely remove the resin from the clogged column and resuspend it. Transfer the resin into a fresh column.
- centrifuge the sample at 12,000×g for 20–30 min at 4 °C and / or filter the sample through a 0.45 µm filter before applying it to the column.

Protein has precipitated in the column:

- regenerate the ProCatch Glutathione Resin (see 3.6)

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## 6. References

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## 7. Appendix

### 7.1 Compatible reagents

Reagent	Acceptable concentration
β-Mercaptoethanol (ME)	10 mM
EDTA/EGTA	10 mM
Ethanol	70% (regeneration)
Guanidinium-HCl	6 M (regeneration)
NaOH	100 mM (regeneration)
Citrate buffer, pH 4	100 mM (regeneration)
NaCl	1 M
Imidazole	200 mM, pH 7.0–8.0
MES	20 mM
MOPS	50 mM
HEPES	50 mM
Tris	50 mM
NP-40	1%
Triton X-100	1%
KCl	500 mM
Glycerol	20%

### 7.2 Protease inhibitors

Inhibitor	Final concentration	Stock solution*
Aprotinin	0.3 μM	1 mg/mL (= 500×) in H <sub>2</sub> O (pH 7)
E-64	10 μM	0.36 mg/mL (= 100×) in 1:1 mixture H <sub>2</sub> O:EtOH (pH 7)
Leupeptin	10 μM	5 mg/mL (= 1000×) in H <sub>2</sub> O (pH 7)
PMSF	1 mM	17 mg/mL (= 100×) in EtOH, isopropanol or methanol; inactivated by DTT

\* Store stock solutions at –20 °C (except for PMSF: store at room temperature).

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