Amicon® Pro Affinity Concentration Kit - GST

Purification of GST-tagged recombinant proteins.

Catalog Nos. ACR5000GS, ACK5003GS, ACK5010GS, ACK5030GS, ACK5050GS, ACK5100GS

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Not for use in diagnostic procedures.

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Introduction

The expression and purification of recombinant proteins is central to protein regulation, structure and function studies. Purification has been greatly facilitated by the addition of small affinity tags to protein sequences such as the glutathione S-transferase domain (GST). GST agarose resin is an affinity chromatography matrix, when used in concert with the Amicon® Pro Affinity Concentration device, offers rapid purification of GST fusion proteins, native GST, or glutathione-binding proteins. GST-tagged proteins bind specifically to reduced glutathione in near-neutral, non-denaturing conditions (e.g., phosphate buffer). Following resin capture of the target protein, unbound lysate components are removed by spin-based clearing and washing steps. Bound protein is recovered by centrifugation following competitive displacement with buffer containing free, reduced glutathione.

By condensing the entire purification workflow into one device, the Amicon® Pro device eliminates the need for multiple sample transfers thereby minimizing protein loss. The large exchange device reservoir (up to 9ml) accommodates a range of sample capacities as well as reduces the need for multiple spin steps during the wash and elution phases. Direct coupling to an Amicon® Ultra-0.5 device further provides simultaneous concentration during the elution phase. Lastly, the Amicon® Pro device offers highly efficient diafiltration in a single 15 minute spin. The kit contains sufficient GST resin, optimized buffers, and Amicon® Pro devices for 12 standard reactions.

Sample Preparation Guidelines

- Optimizing lysis parameters is critical for protein extraction and downstream purification. The Amicon® Pro Affinity Concentration GST kit is compatible with a range of conditions, including reducing agents (β-mercaptoethanol, DTT), chelating agents (EDTA), non-ionic detergents, and BugBuster® Protein Extraction Reagent (Cat. No. 70584), a proprietary mixture of non-ionic detergents offering a rapid cost-effective alternative to mechanical cell lysis methods such as sonication.
- Irrespective of extraction method, we recommend inclusion of rLysozyme™ Solution (Cat. No. 71110) and Benzonase® Nuclease (Cat. No. 70746) and during protein extraction for increased cell lysis (protein yield) and a reduction in sample viscosity (improved sample handling), respectively.
- Protease inhibitors may also be added to the lysis buffers to protect against degradative enzymes. A listing of individual protease inhibitors and cocktails is available at http://www.emdmillipore.com/ search on key words "***Protease Inhibitor***." Note: Serine proteases should be used with caution if the GST fusion tag is to be cleaved via Thrombin or Factor Xa digestion. If used during protein extraction, we strongly recommend the eluted fraction be buffer exchanged prior to performing the cleavage reaction.
- In certain systems, high protein expression can lead to aggregation in the form of inclusion bodies. Strong denaturants such as 6 M guanidine or 8 M urea can be used to solubilize aggregates greatly enhancing yield. However, only properly folded, functional GST is capable of binding GST resin. GST fractions recovered from denaturation of inclusion bodies must be refolded to reconstitute active GST fusion constructs. To a degree, this can be accomplished through use of the Protein Refolding Kit (Cat. No. 70123).

- For more information, consult the GST•Bind™ Resin User Guide available at http://www.emdmillipore.com/chemicals. Search using the Cat. No. 70541.

Kit Components

- CS211421-GST Resin (3 mL) – Crosslinked agarose with immobilized reduced glutathione supplied as 50% slurry in 50 mM Phosphate Buffer pH 7.5, 0.15 M NaCl, 0.1% NaN_3. The resin utilizes an 11-atom spacer arm (~16 Å) for covalent attachment of reduced glutathione via a sulfide linkage. The binding capacity is typically 5-8 mg/mL of settled resin.
- Buffers:
  - CS211416-10X GST Bind/Wash Buffer (25 mL) – 43 mM Na_2HPO_4, 14.7 mM KH_2PO_4, 1.37 M NaCl, 27 mM KCl pH7.2
  - CS211354-10X Glutathione Reconstitution buffer (5 mL) – 500 mM Tris, pH 8.0
  - CS211418-Glutathione, reduced, free acid (125 mg)
- Amicon® Pro Devices – The kit includes 12 complete assemblies. Each device consists of an exchange device, holder tube, 50 mL collection tube, and Amicon® Ultra-0.5 centrifugal filter device. A 2 mL collection tube is included for sample recovery from the AU-0.5 device by reverse spin. The kit is available in five formats based on the nominal molecular weight limit (NMWL) of the AU-0.5: 3 (ACK5003GS), 10 (ACK5010GS), 30 (ACK5030GS), 50 (ACK5050GS), and 100 kDa (ACK5100GS). Consult the Amicon® Pro (http://www.emdmillipore.com/psp, search keywords "Amicon Pro") and Amicon® Ultra-0.5 centrifugal filter device User Guides (http://www.millipore.com/catalogue/module/c82301) for proper assembly/disassembly and additional product information.

All reagents should be stored at 2° to 8°C (do not freeze). The Amicon Pro devices can be stored separately at room temperature.

Procedures for using the Amicon® Pro Affinity Concentration Kit - GST

The protocol is based on purification of GST-tagged protein from 1 mL of *E. coli* lysate using 200 µl of GST resin slurry (100 µL packed resin). The protocol is linearly scalable for 50-1000 µL of resin slurry. Due to large variability among sample preps, parameters which may require optimization include bead input, binding time, wash, and elution parameters. This protocol includes steps for simultaneous concentration during the elution step as well as buffer exchange using the Amicon® Ultra-0.5 centrifugal filter device.

Note: Given the collection tube's capacity, it is not necessary to remove filtrate between the various centrifugation steps. However, if process samples need be retained for analytical purposes, the collection tube should be cleared.

Buffer Preparation

- 10X Bind/Wash Buffer should be diluted to 1X with sterile deionized H_2O; 1X Bind/Wash solution may be stored at 4°C for 1 week.
- Prepare 10X GST Elution Buffer containing 100 mM reduced glutathione as follows: add 4ml 10X Reconstitution buffer directly to the glutathione powder vial. Pipette repeatedly then incubate for 30 minutes at room temperature. Divide buffer into working volumes and store at -20°C (400

µl/standard reaction); 10X GST Elution Buffer is stable for 6 months at -20°C. We do not recommend repeated freeze/thaw cycles. To minimize glutathione oxidation, dilute 10X buffer to 1X using sterile deionized water and pH to 8.0 immediately before use. Discard any unused 1X Elution Buffer.

Bead Preparation

1. To ensure uniform suspension, vortex the GST resin thoroughly before adding it to the device.
2. Remove the collection tube cap and open the exchange device cap.
3. Add 200 µL of resin slurry to the base of the exchange device. Close the exchange cap.
   - Up to 500 µl packed resin (1000 µl slurry volume) may be added per device We recommend using wide-bore tips (Cat. No. 02-707-134, Fisher Scientific) for resin transfer.
4. To remove storage buffer, centrifuge in a swinging bucket rotor at 1000 g X 1 min.
5. Add 500 µL of 1X Bind Buffer. Centrifuge at 1000 g X 1 min.

Protein Binding

1. Add 500 µL of sample to the exchange device.
   - Up to 9 mL of sample can be added. The volume loaded is determined by the target protein's expression level and resin's binding capacity.
2. Incubate for 60 min at room temp with gentle agitation.
   - We recommend upright agitation on a plate shaker at low setting.
   - End-over-end mixing, particularly with small volumes or for extended time, may result in substantial bead loss to the sides of the feeder tube.
   - The duration of binding time may vary with application.
3. Centrifuge the device at 1000 g X 1 min in a swinging bucket rotor. Recover the sample flow-through from the 50 mL collection tube (optional).
   - To ensure maximal protein capture, collect all resin into solution prior to centrifugation.
4. Add1.5 mL of Wash Buffer. Centrifuge at 1000 g X 1 min. Recover the wash fraction from the 50 mL collection tube (optional).
   - Due to the large capacity of the exchange device, the volume of the wash can be increased for greater sample purity. There is no need for multiple wash steps.

Sample Elution

Samples can be eluted without concentration by adding elution buffer and centrifuging (1000g X 2 minute) directly into a clean 50 ml collection tube. Given the limited volume processing capacity of the AU-0.5 device, we recommend this protocol if elution volumes > 1.5 ml are required.

For simultaneous elution with concentration, attach the Amicon® Ultra-0.5 device and follow the steps outlined below.

1. Remove the exchange device and insert it into the AU0.5 device.
2. Place the exchange device/AU-0.5 assembly back in the holder and return the device to the collection tube.
3. Add up to 1.5 mL of Elution Buffer, gently resuspend the resin, and incubate for 5 min.
   - Under standard conditions, one elution is sufficient for recovery of 90-95% of captured protein.
   -
4. Close the exchange device cap and screw on the collection tube cap to ensure a proper seal.
5. Centrifuge at 4000 g X 15 min in a swinging bucket rotor. Concentrated samples can be buffer exchanged or recovered from the AU-0.5 device by reverse spin (see below).

- Depending on the starting elution volume, NMWL of AU0.5 device employed, and the degree of concentration desired, the length of the spin time can range for 10-30 minutes. Please consult the **Performance Characteristics** section in the Amicon® Pro Affinity Concentration System User Guide (http://ww.emdmillipore.com/psp, and search keywords "Amicon Pro") for recommended guidelines.
- , consult.

6. Recover the concentrated fraction by reverse spin or proceed to Buffer Exchange (see below).

Buffer Exchange (Optional if samples have been collected in the Amicon® Ultra-0.5 device)

1. After sample concentration, add 1.5ml desired buffer to the exchange device/AU-0.5 assembly.
2. Centrifuge device at 4000g X 15 minutes in a swinging bucket rotor. Concentrated samples can be recovered from the AU-0.5 device by reverse spin (see below).

Collect sample from the AU0.5 device by Reverse Spin (following Concentration or Buffer Exchange)

1. Disassemble the exchange device/AU-0.5 assembly from the holder tube.
2. Using a gentle twisting motion, detach the AU-0.5 from the exchange device.
3. If there is residual sample in the exchange device tip, depress the exchange device cap to expel the remaining sample volume into the AU-0.5.
4. Hold AU-0.5 upright and slide the 2 ml collection tube on top of it.
5. Invert the assembly and centrifuge (in a microcentrifuge) with a fixed angle rotor 1000g X 2min.

Sample protein yield can be determined by Mid IR-based spectrometry using the DirectDetect™ biomolecular quantitation system and DirectDetect™ Assay Free Sample Cards.

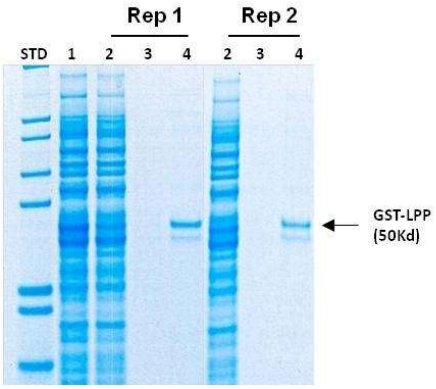
Troubleshooting

Issue: Recombinant Protein is present in low amount in eluate	
Possible Cause	**Solution**
Protein is insoluble and may have formed inclusion bodies.	After lysate clearance, check both the supernatant and pellet for protein. Perform lysis and binding procedures under denaturing conditions.
The GST fusion protein is not in the proper three dimensional conformation.	Attempt to reconstitute native protein structure using Protein Refolding Kit (Cat. No. 70532).
The GST-tag is not exposed for binding to the affinity resin.	The protein may require denaturing conditions for binding.
The GST-tag is not present.	Sequence the ligation junctions to ensure that the reading frame is correct. Check for possible internal translation starts (N-terminal tag) or premature termination sites (C-terminal tag).
Recombinant protein is degraded during cell lysis.	Add protease inhibitors during cell lysis.
Protein forms aggregates.	Add solubilizing agents such as detergents (0.1% Triton® X-100, TWEEN®-20) or increase salt concentration.
pH of the Lysis or Binding buffers is incorrect.	Check buffer pH; the acceptable range is 7-8. Acidic buffers will prevent binding.
Protein expression is insufficient.	Optimize the growth/induction conditions.
Cell Lysis is incomplete.	Optimize the Cell Lysis Protocol.
Cell Lysate is too viscous.	If possible, dilute the lysate in Bind Buffer. Alternatively, include Benzonase® Nuclease during lysis to remove free RNA/DNA.
Protein precipitates during sample concentration while using the AU0.5 device due to over-concentration.	Reduce the duration of the centrifugation time during the elution/concentration step.
Protein is lost during sample concentration while using AU0.5 device.	Check the protein's expected size and MWCO of AU0.5 device used. AU0.5 device is offered in 5 different MWCO formats - 3, 10, 30, 50, and 100 kDa.

Issue: High Non-specific binding	
Possible Cause	**Solution**
Insufficient washing.	Increase the volume of the Wash Buffer used or the number of wash steps. Alternatively, supplement the Bind/Wash Buffers.
Contaminants interact directly with the GST fusion protein.	Add reducing agents such as DTT or β-mercaptoethanol to reduce disulfide bonds. Add detergents to disrupt non-specific interactions.
GST fusion protein is degraded.	Degraded/truncated forms of the recombinant protein will still bind to the GST resin and appear as contaminating bands in SDS-PAGE. Perform a lysis procedure on ice and include protease inhibitors.
Cell lysate is too concentrated.	Dilute the lysate in Bind Buffer before purification.

Performance

Purification using Amicon® Pro Affinity Concentration Kit – GST



Replicate purifications of a 50 kDa GST-tagged protein from *E. coli* lysate using the Amicon® Pro Affinity Concentration Kit - GST. The numbered lanes are:
1 – lysate
2 – flowthrough fraction
3 – wash fraction
4 – eluted fraction

Product Ordering Information

	No Devices	Amicon® Pro + AU 0.5 ml with MWCO:				
		3k	10k	30k	50k	100k
Amicon® Pro Affinity Concentration Kit - GST	ACR5000GS	ACK5003GS	ACK5010GS	ACK5030GS	ACK5050GS	ACK5100GS
Amicon® Pro Affinity Concentration System 12PK		ACS500312	ACS501012	ACS503012	ACS505012	ACS510012
Amicon® Pro Affinity Concentration System 24PK		ACS500324	ACS501024	ACS503024	ACS505024	ACS510024

The Amicon® Pro Affinity Concentration Kit contains reagents and devices sufficient for 12 standard reactions. Amicon® Pro devices are also sold separately in 12 and 24 packs.

Description	Catalogue Number	Qty/Pack
GST•Bind™ Resin	70541-3/4/5	10/50/25 mL
GST•Bind™ Buffer Kit	70534	1 Kit

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