

Purification of recombinant fusion proteins

Purification of recombinant proteins

Examples

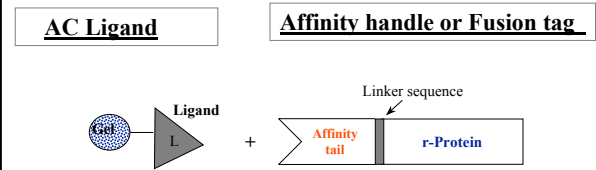
- Fusion protein
 - Soluble in cytoplasm
 - Inclusion bodies
- Non-fusion protein
 - Inclusion bodies
 - Expressed in periplasm

Fusion Proteins for Affinity Purification

Fusion partner	Ligand
• Glutathione S-transferase (GST)*	Glutathione
• Poly-His tag	Chelating
• Protein A/ "Z" domains*	IgG
• Calmodulin-binding peptide (CBP)	Calmodulin
• Biotinylated peptide	Streptavidin/avidin
• Strep-tag II	Modified streptavidin
• Thioredoxin*	Phenylarsine oxide (PAO)
• Chitin-binding domain	Chitin
• Maltose-binding protein (MBP)*	Amylose/dextrin
• Cellulose-binding domain (CBD)	Cellulose
• FLAG epitope	ANTI-FLAG monoclonal antibodies
• T7-tag	T7-Tag monoclonal antibody
• Protein C epitope	Anti-Protein C monoclonal antibody
• HA peptide epitope	Anti-HA High Affinity monoclonal antibody
• S-Tag	S-protein

Bolded ligands attached to Sepharose™ are from Amersham Pharmacia Biotech.
 Note: CNBr- and NHS- activated Sepharose bind other ligands.
 * Tags known to improve the solubility of the fusion protein.

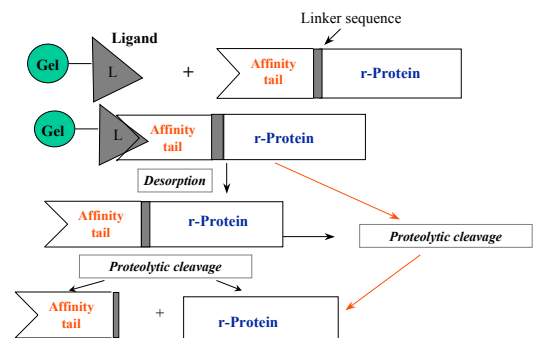
Genetic manipulations



Genetic manipulations

Affinity handle or Fusion tag	AC Ligand
• Glutathione S Transferase (GST)	• Glutathione
• Poly Histidine	• Nickel ions
■ Maltose Binding Protein (MBP)	■ Amylose/anti IgG
❖ E tag sequence	❖ Anti E IgG
✱ ZZ (domain B of protein A)	✱ IgG
◆ Protein A	◆ IgG

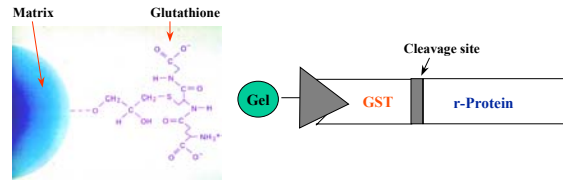
Purification of fusion proteins



GST gene fusion system

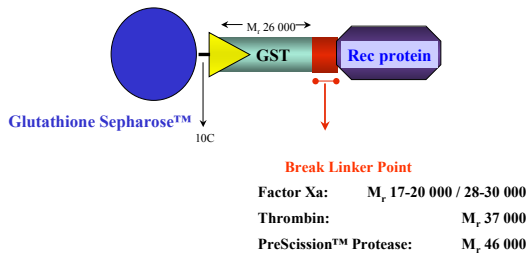
- **Integrated system for**
 - expression
 - purification
 - detection of glutathione S- transferase fusion proteins in *E. coli*
- **Includes**
 - choice of ten GST expression vectors
 - purification module
 - detection module

GST fusion protein



- Glutathione S Transferase (GST), 26 kD
- GST is fused via its C terminus to the target protein
- Binds to Glutathione Sepharose 4B even in 8M Urea or 6M Guanidine HCl
- Thrombin, Factor Xa and PreScission™ Protease cleavage site at C terminus of GST
- GST activity is easily assayed.

Purification of GST Fusion Proteins

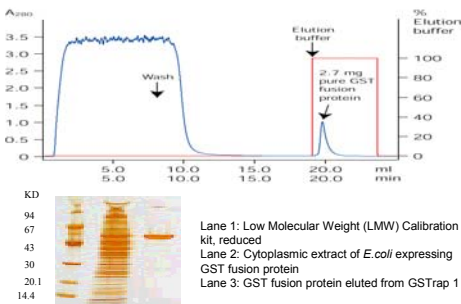


Simple Purification at Any Scale

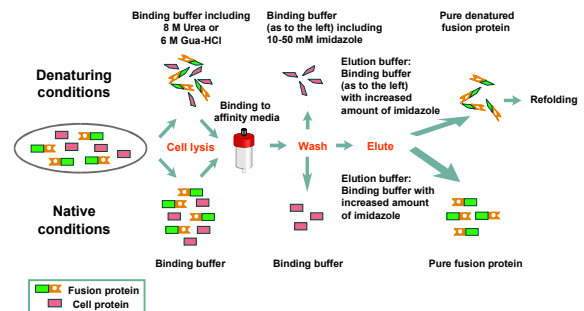
Column/or media	Amount of GST fusion protein for a single purification	Comment
GST MicroSpin™ Purification Module	Up to 400 µg	Ready to use, pre-packed columns, buffers and chemicals High throughput when used with MicroPlex™ 24 Vacuum (up to 48 samples simultaneously)
GSTrap™ 1 ml	10 - 12 mg	Pre-packed column, ready to use
GSTrap 5 ml	50 - 60 mg	Pre-packed column, ready to use
Glutathione Sepharose™ 4B	8 mg/ml	For packing small columns and other formats
Glutathione Sepharose 4 FF	10 -12 mg/ml	For packing high performance columns for use with purification systems and scaling up

Purification of recombinant GST-tagged protein

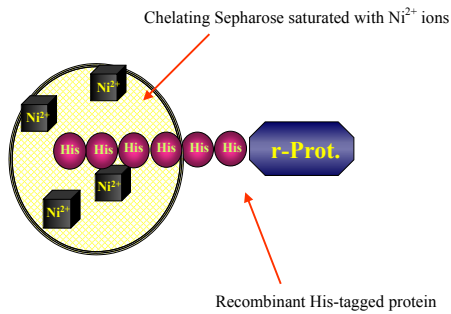
Sample: 8 ml cytoplasmic extract from *E. coli* expressing a GST fusion protein
 Column: GSTrap 1 ml
 Binding buffer: PBS, pH 7.3
 Elution buffer: 50 mM Tris-HCl, pH 8.0 with 10 mM reduced glutathione
 System: AKTAexplorer 10, 1 ml/min



General Purification of fusion proteins



Purification and Detection of (His)₆ Fusion Proteins



Simple Purification at Any Scale

Column/or media	Amount of GST fusion protein for a single purification	Comment
GST MicroSpin™ Purification Module	Up to 400 µg	Ready to use, pre-packed columns, buffers and chemicals High throughput when used with MicroPlex™ 24 Vacuum (up to 48 samples simultaneously)
GSTrap™ 1 ml	10 - 12 mg	Pre-packed column, ready to use
GSTrap 5 ml	50 - 60 mg	Pre-packed column, ready to use
Glutathione Sepharose™ 4B	8 mg/ml	For packing small columns and other formats
Glutathione Sepharose 4 FF	10 -12 mg/ml	For packing high performance columns for use with purification systems and scaling up

His MicroSpin™ Purification Module

For screening, optimization of protein amplification and small scale purification



- Ready to use, 50 pre-packed columns with buffers and chemicals
- Purify up to 100 µg His tagged protein per column

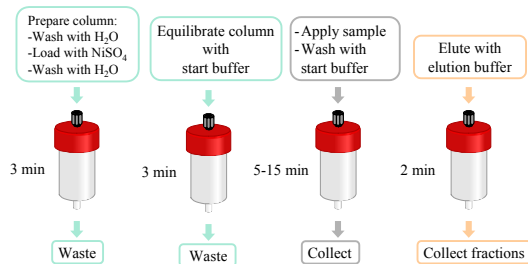
HisTrap™ Kit

For speed, reproducibility and ease of use



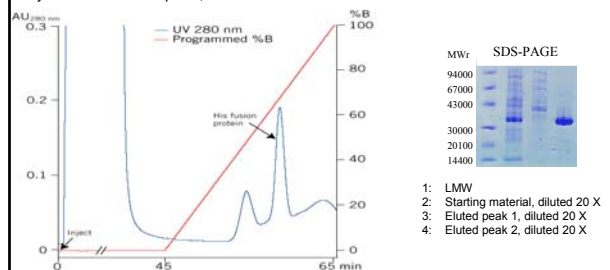
- Purify up to 12 mg per HiTrap™ Chelating HP column in less than 25 min
- Includes all necessary buffers and accessories
- Use with syringe, peristaltic pump or connected to a system (e.g. ÄKTA™prime)
- Compatible with additives

Simple Purification on HiTrap™ HP Chelating

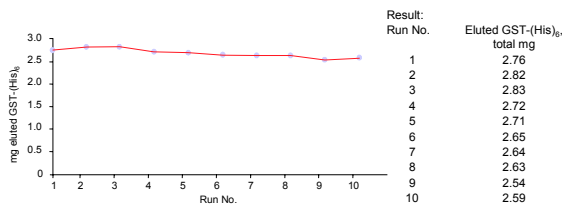


Purification of recombinant His-tagged protein

Sample: Clarified homogenate of *E. coli* expressing His fusion protein
 Column: HiTrap Chelating 1 ml charged with Ni²⁺
 Binding buffer: 20 mM sodium phosphate, 0.5 M sodium chloride, 10 mM imidazole, pH 7.4
 Elution buffer: 20 mM sodium phosphate, 0.5 M sodium chloride, 0.5 M imidazole, pH 7.4
 System: ÄKTAprime, 1 ml/min



10 repetitive purifications of a (His)₆ fusion protein without metal reloading



Samples: 2.5 ml GST-(His)₆ cell extract
Binding buffer: 20 mM Na phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4
Elution buffer: 20 mM Na phosphate, 0.5 M NaCl, 500 mM imidazole, pH 7.4
Flow: 2 ml/min, 312 cm/h
Note: No Ni²⁺ reloading of the column between the runs

Chelating Sepharose™ Fast Flow

For packing high performance columns used with purification systems and for scaling up

- Purify up to 12 mg per ml of medium
- Use with XK columns connected to an ÄKTA™ design system
- Run at high flow rates
- Compatible with additives

Detection of (His)₆ Fusion Proteins

Detection method	Comments
ELISA assay using anti-His antibody	Highly specific, detects only (His) ₆ fusion protein
Functional assays	Useful to assess if the purified (His) ₆ fusion protein is active. Not always available May require development and optimization

Detection of (His)₆ Fusion Proteins

Detection method	Comments
Western blot analysis using anti-His antibody and ECL™ detection systems.	Highly specific, detects only (His) ₆ fusion protein. Little or no background when used at optimized concentrations with secondary HRP conjugated antibody. ECL detection systems enhance detection in Western blot. ECL provides adequate sensitivity for most recombinant expression applications. For higher sensitivity use ECL Plus™.
SDS-PAGE with Coomassie™ Blue or silver staining.	Provides information on size and % purity. Detects fusion protein and contaminants.



*Thank you very much
for your attention!*