

# **Analytical Biochemistry**

## **3570**

# **Affinity chromatography**

## GST gene fusion system (Pharmacia)

The Glutathione S-transferase (GST) Gene Fusion System is an integrated system for the expression, purification and detection of fusion proteins produced in *E. coli*. The system consists of three major components: pGEX plasmid vectors, two GST Purification Modules and the GST Detection Module. The pGEX plasmids are designed for inducible, high-level intracellular **expression of genes** or gene fragments **as fusions with *Schistosoma japonicum* GST**. Fusion proteins are easily purified from bacterial lysates by **affinity chromatography using Glutathione Sepharose 4B** contained in the GST Purification Modules. Cleavage of the desired protein from GST is achieved using a *site-specific protease whose recognition sequence is located immediately upstream from the multiple cloning site* on the pGEX plasmids. Fusion proteins can be detected using a colorimetric assay or immunoassay provided in the GST Detection Module.

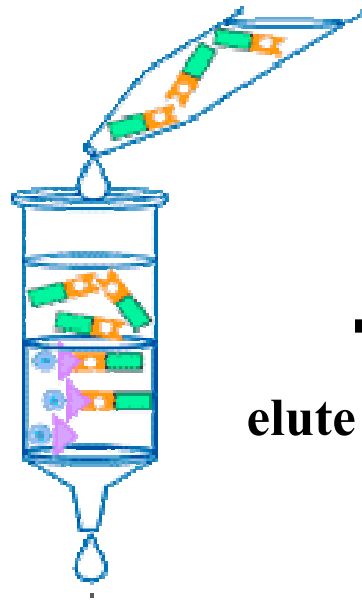


# Crude lysate containing GST-tagged recombinant proteins

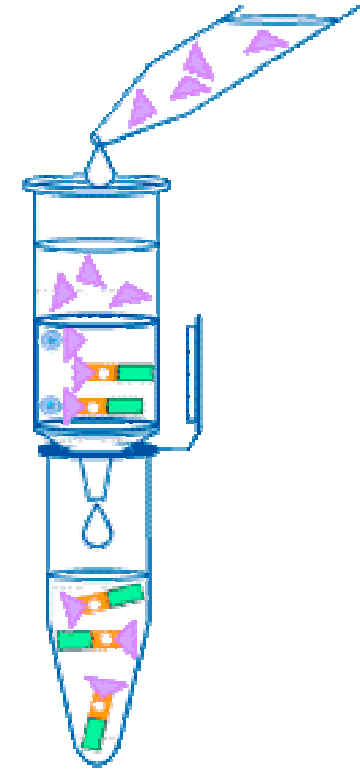
 recomb. prot.

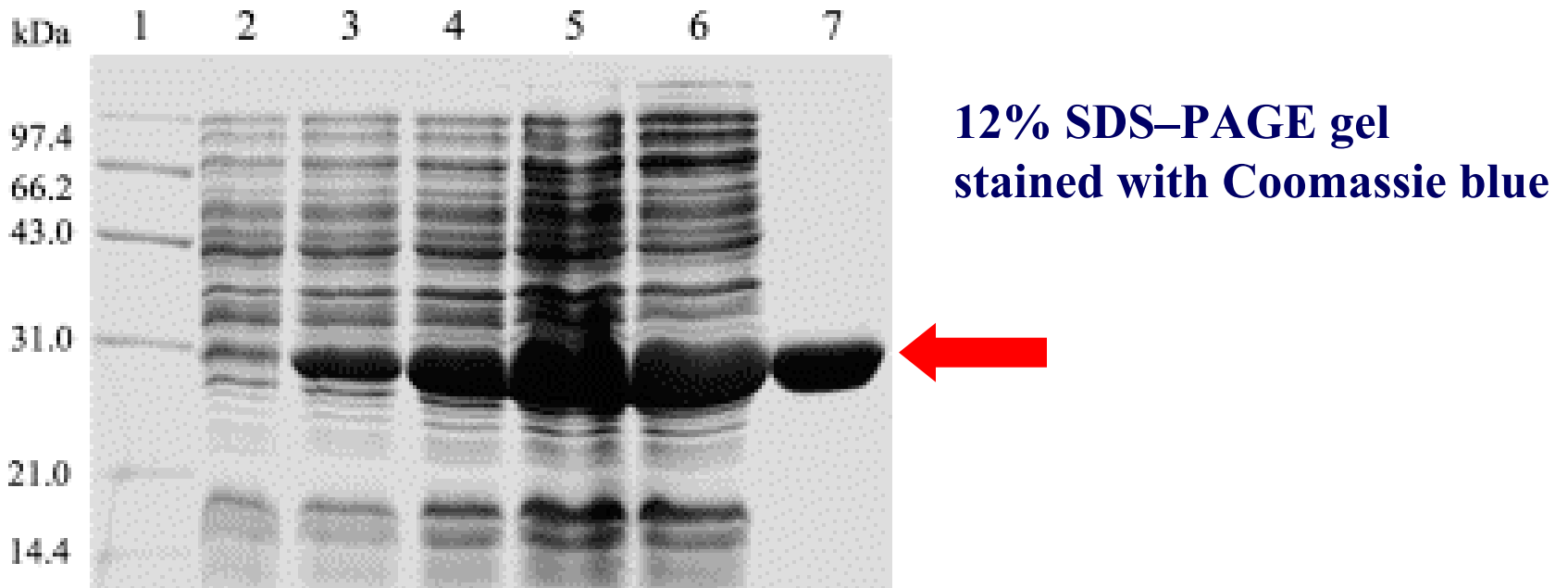
 GST

  
GSH-Sepharose  
column



elute with GSH





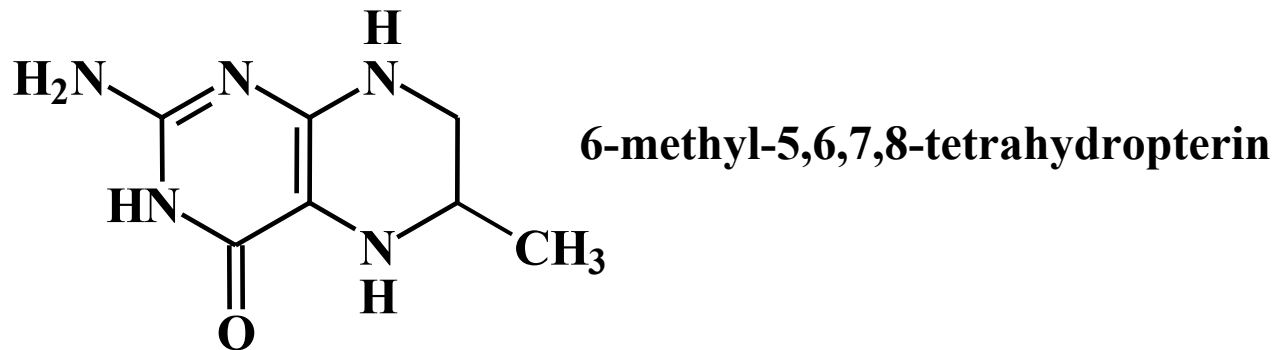
### SDS-PAGE analysis of fusion protein

- 1: molecular weight markers;
- 2: bacterial total soluble protein, no induction;
- 3–6: total soluble protein, IPTG induction for 1–4 h;
- 7: fusion protein purified by GSH–Sepharose 4B affinity chrom.

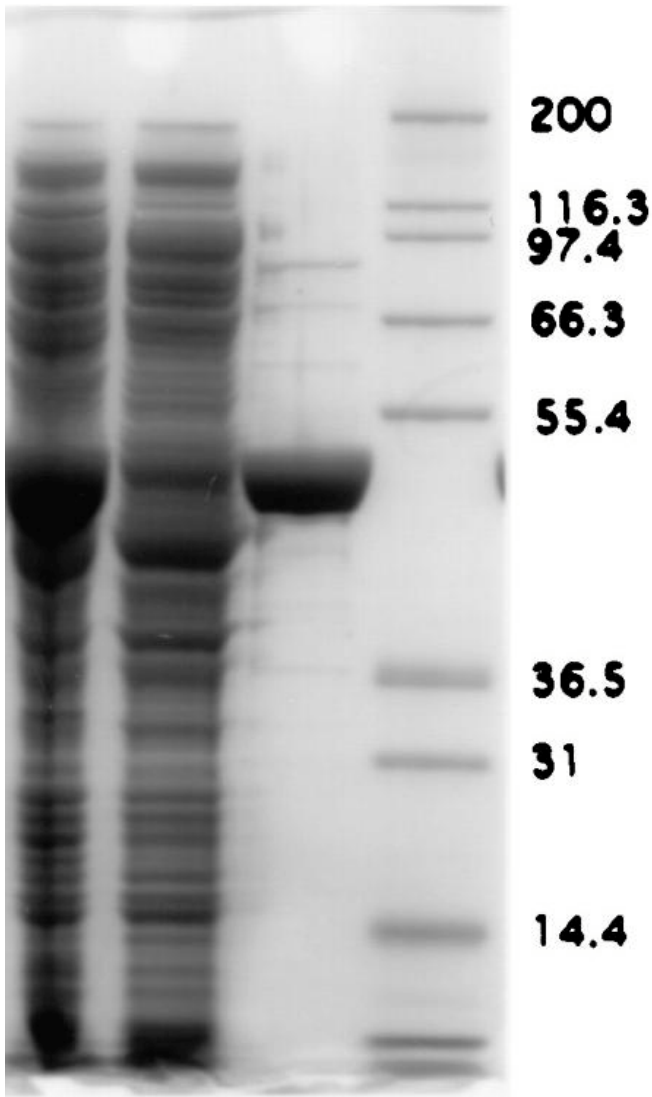
Zhang *et al.*, Expression, purification, and C-terminal amidation of recombinant human glucagon-like peptide-1, *Protein Expr. Purif.* 36: 292-9, 2004.

**Wang *et al.*, Mutagenesis of the regulatory domain of phenylalanine hydroxylase, *Proc. Natl. Acad. Sci. U.S.A.* 98: 1537-1542, 2001**

**Phenylalanine hydroxylase (PAH) catalyzes the pterin-dependent hydroxylation of phenylalanine to tyrosine. ...**



(structure is “for information only”)



We chose to use an affinity chromatographic approach with a pterin-conjugated Sepharose column ... made by conjugating 6-methyl-5,6,7,8-tetrahydropterin to a carboxy group on a solid phase. ... The purification is then very simple, and pure rat PAH can be obtained at high concentration in just one step from the crude extract.

Expression and purification of rat PAH shown on 10% SDS/PAGE gels. Gels were stained with colloidal blue staining kit.

*From left:* crude *E. coli* extracts; flow-through after applying crude extract; eluate with  $\text{NaHCO}_3/\text{NaOH}$  (pH 10.8); protein-standard markers.