- Centrifuge the suspension at 500 x g for 5 minutes to sediment the matrix. Discard the wash.
- Repeat the wash twice for a total of three washes.
- To the sedimented matrix, add 1.0 ml of Elution Buffer (10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0) per ml bed volume of Glutathione Sepharose 4B.
- Mix gently to resuspend the matrix. Incubate at room temperature (22-25 °C) for 10 minutes to elute the bound material from the matrix.
- Centrifuge at 500 x g for 5 minutes to sediment the matrix, and remove the supernatant. Save in a fresh centrifuge tube.
- Repeat elution and centrifugation steps twice more. Pool the three eluates.

Note: Following elution steps, a significant amount of GST fusion protein may remain bound to the matrix. Volumes and times used for elution may vary among GST fusion proteins. Additional elutions may be required. Eluates should be monitored for GST fusion protein by SDS-PAGE or by CDNB assay (GST Detection Module, Code No. 27-4590-01).

Note: The yield of a GST fusion protein can be estimated by measuring the absorbance at 280 nm. For GST, 1 $A_{280} \approx 0.5$ mg/ml.

Companion Products

	Code No.	Pack Size
Bulk GST		
Purification Module	27-4570-01	5 purifications
RediPack GST		
Purification Module	27-4570-02	2 purifications
GST Detection Module	27-4590-01	50 reactions
pGEX GST Gene Fusion Vectors		
see Pharmacia's Molecular and Cell Biology Catalogue		

Pharmacia Biotech

Printed in Sweden by TK i Uppsala, November, 1994

Glutathione Sepharose® 4B *INSTRUCTIONS*

Glutathione Sepharose 4B is designed for rapid single step purification of recombinant derivatives of glutathione S-transferase, other glutathione S-transferases or glutathione dependent proteins. The gel is also available as pre-packed disposible columns (Code No. 17-0757-01) for rapid and convenient purification of smaller quantities of proteins.

Gel characteristics

Content: 10 ml Glutathione Sepharose 4B in 20% ethanol as preservative.

Degree of substitution: 200-400 µmol Glutathione Sepharose per g dried substance.

Bead form: Spherical, 45-165 µm wet bead diameter.

Spacer arm: 12 atoms (10 carbons)

Chemical stability: No significant loss of the capacity is detected when Glutathione Sepharose 4B is exposed to 0.1 M acetate pH 4.0, 0.1 M NaOH, M 70 ethanol or 6 M guanidine hydro-chloride for 1 hour at room temperature.

Note: It is not recommended to autoclave the gel.

52-2303-00
Edition AE

Pharmacia Biotech

GIBCOBRL

0.24-9.5 Kb RNA Ladder

Cat. No. 15620-016

Lot No. FM3701 75 µg; 1 µg/µl

STORE AT -20°C. (AVOID FROST FREE)

Description:
The 0.24-9.5 Kb RNA Ladder contains a mixture of six synthetic poly(A)-tailed RNAs. Each RNA contains sequences derived from bacteriophage T7, yeast 2 µ circle and bacteriophage λ DNA. This ladder is suitable for sizing single-stranded RNA from 0.24 to 9.5 kb in glyoxal or formaldehyde gels. The bands can be stained with ethidium bromide or detected on Northern blots with λ DNA probes (1). One 3-µl application of ladder contains approximately 0.5 µg of each component.

Component:
15620-016 0.24-9.5 Kb RNA Ladder Lot No. FM3701

Storage Buffer:
10 mM HEPES (pH 7.2)
2 mM EDTA

Notes:
To avoid damaging the Ladder through repeated freeze thaw cycles or adventitious RNase contamination, it is recommended that the 0.24-9.5 Kb RNA Ladder be aliquoted into single use quantities. The 0.24-9.5 Kb RNA Ladder contains no SDS or other RNase inhibitor. Extreme care is needed during manipulations to prevent trace RNase contamination. Gloves should always be worn. Whenever possible, solutions should be pretreated with diethylpyrocarbonate (DEPC) and autoclaved. Tubes, tips and glassware should be pretreated with DEPC, autoclaved for 45 minutes and baked dry.

To use, thaw the ladder on ice and mix thoroughly but gently. Do not vortex. While thawed, keep on ice.

Doc. Rev.: 042195

Instructions for use

- Pack the gel in any suitable Pharmacia chromatography column.
- Wash the column with 5-10 bed volumes of PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) to remove the preservative.
- Equilibrate the gel bed with 3 bed volumes PBS.
- If needed, clarify the sample by centrifugation and filter the supernatant through a 0.45 µm filter before applying to the column. The sample may be diluted in PBS + 1% Triton X-100, if too concentrated.
- Apply the sample to the column and discard the eluent.
- Wash the column with 5-10 bed volumes of PBS.
- Elute the bound material with 5 bed volumes of elution buffer (10 mM Glutathione in 50 mM Tris-HCl pH 8.0) and collect the fractions.

Regeneration and Storage

Glutathione Sepharose 4B may be regenerated for re-use by washing the gel with 2–3 bed volumes of alternating high pH (0.1 M Tris-HCl + 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate + 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration with 3–5 bed volumes of 1X PBS.
If the gel appears to be loosing binding capacity, it may be due to an accumulation of precipitated, denatured or non-specifically bound proteins.
To remove precipitated or denatured substances, wash the matrix with 2 bed volumes of 6 M guanidine hydrochloride, immediately followed by a wash with 5 bed volumes of 1X PBS.
To remove hydrophobically bound substances, wash the matrix with 3–4 bed volumes of 70% ethanol or with 2 bed volumes of a non-ionic detergent (conc. 0.1%), immediately followed by a wash with 5 bed volumes of 1X PBS.
For long-term storage (> 1 month) the following procedure of additional washes is recommended:

- Wash the gel twice with 10 bed volumes of 1X PBS.
- Repeat washes using 20% ethanol.
- Store at +4 °C.
- Re-equilibrate the gel with 1X PBS before re-use.

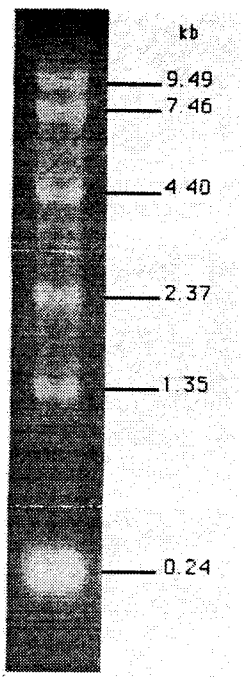
Instructions for batch purifications

Preparation of Matrix

- Gently shake the bottle of Glutathione Sepharose 4B to resuspend the matrix.
- Use a pipet to remove sufficient slurry for use and transfer to an appropriate container/tube. [Glutathione Sepharose 4B as supplied is approximately a 75% slurry. The following procedure results in a 50% slurry. Dispense 1.33 ml of the original Glutathione Sepharose 4B slurry per ml of bed volume required. 1 ml of bed volume will bind approximately 5 mg of glutathione S-transferase (GST).]
- Sediment the matrix by centrifugation at 500 x g for 5 minutes. Carefully decant the supernatant.
- Wash the Glutathione Sepharose 4B by the addition of 10 ml of cold (4 °C) PBS per 1.33 ml of the original slurry of Glutathione Sepharose 4B dispensed. Invert to mix.
- Sediment the matrix by centrifugation at 500 x g for 5 minutes. Decant the supernatant.
- For each 1.33 ml of the original slurry of Glutathione Sepharose 4B dispensed, add 1 ml of 1X PBS. This results in a 50% slurry. Mix well prior to subsequent pipetting steps.
  Note: Glutathione Sepharose 4B equilibrated with PBS may be stored at 4 °C for up to 1 month.

Batch purification of glutathione S-transferase proteins

- Add 2 ml of the 50% slurry of Glutathione Sepharose 4B equilibrated with PBS per 5 mg of GST in PBS + 1% Triton X-100. (If you are purifying a GST fusion protein produced from a pGEX vector, a typical yield is approximately 2.5 µg GST fusion protein/ml culture).
- Incubate with gentle agitation at room temperature for 30 minutes.
- Centrifuge the suspension at 500 x g for 5 minutes to sediment the matrix. Remove the supernatant.
- Wash the Glutathione Sepharose 4B pellet with 10 bed volumes of PBS (bed volume = 0.5 x the volume of 50% Glutathione Sepharose slurry used.)



0.24-9.5 Kb RNA Ladder
3 µg/lane (formaldehyde-treated)
1.2% agarose gel
stained with ethidium bromide

Quality Control Data:
When electrophoresed, the formaldehyde treated 0.24-9.5 Kb RNA Ladder clearly exhibits six distinct bands of the expected sizes and of similar intensity. 240 bp band is brighter for orientation.

Reference:
1.Crouse, J. and Amorese, D. (1986) *Focus 8:3*, 8.

Recommended Procedure:

Formaldehyde Gels

1. Denature 3 µg of 0.24-9.5 Kb RNA Ladder in 2.2 M formaldehyde, 50% (v/v) deionized formamide, 50 mM MOPS (pH 7.0), 1 mM EDTA for 10 min at 70°C. Quench on ice.
2. Load with 0.02% (w/v) xylene cyanol, 0.02% (w/v) bromophenol blue onto a 10 min pre-electrophoresed horizontal 1.2% agarose, 2.2 M formaldehyde, 50 mM MOPS (pH 7.0), 1 mM EDTA gel submerged in 50 mM MOPS (pH 7.0), 1 mM EDTA. Electrophorese at 100 V until bromophenol blue dye has migrated two-thirds the length of the gel.
3. In the dark, stain gel 10 min in 5 µg/ml EtBr in distilled deionized water; destain 10 min. in distilled deionized water. Smaller bands of 0.24-9.5 Kb RNA Ladder may diffuse out of the gel during longer overnight destaining. Alternatively, an RNase-free solution of EtBr may be added to each sample to a final concentration of 1 mg/ml, making staining and destaining unnecessary.
4. Photograph gel by short-wave UV transillumination (300 nm) with an orange filter and high-speed film at f4.5 for 1/8 s. Expose gel to UV light only during the time of film exposure. Extended exposure will cause the RNA bands to fade and disappear.

Glyoxal Gels

1. Denature 3 µg of 0.24-9.5 Kb RNA Ladder in 1 M glyoxal (deionized), 50% (v/v) DMSO, 10 mM sodium phosphate (pH 7.0) for 1 h at 50°C.
2. Load with 0.01% (w/v) xylene cyanol, 0.01% (w/v) bromophenol blue onto a horizontal 1.2% agarose, 10 mM sodium phosphate (pH 7.0), 2 mg/ml sodium iodoacetate gel submerged in 10 mM sodium phosphate (pH 7.0). Electrophorese 2 h at 160 V. Circulate buffer to maintain neutral pH.
3. In the dark, stain gel 15 min in 0.5 M ammonium acetate, 5 µg/ml EtBr; destain 15 min in 0.5 M ammonium acetate. Avoid exposing stained gel to light.
4. Photograph as formaldehyde gel using short wave UV transillumination.

NOTE: Bottom band may not be visible on glyoxal gel.