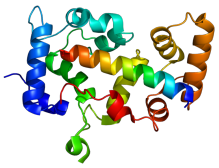
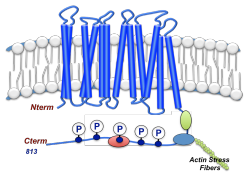


Wash, Strip and Reload Glutathione (GST) Beads Protocol



INTRODUCTION

From GE 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.

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 (TX100 will work at a 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.