Purification of GST-MS2 (2 Liter preparation)

GST-MS2 (glutathione-S-transferase) fusion protein is highly expressed but readily forms insoluble aggregates. The following protocol has been found to maximize solubility.

1. The plasmid is transformed into BL21-DE3 cells (invitrogen)
2. Inoculate a 50ml overnight culture in LB supplemented with 100ug/ml ampicillin
3. At around 2pm, 2 liter of LB Broth supplemented with 100ug/ml ampicillin are inoculated with 10ml of overnight culture per liter (1:100 dilute)
4. Grow to A600 of 0.6 at 37℃ (~2h15min).
5. Cool to room temperature and add 0.1mM IPTG.
6. Growth is continued overnight at room temperature.
7. Pellet the induced cultures at 5000g for 10min and resuspend in 50ml of lysis buffer
8. Lysed in a French press (Thermo Electron) by passing through twice. (or sonicate at 60% intensity for 1-1.5min, 8sec pulse + 1min break)
9. The lysate is clarified twice by centrifugation for 30min at 13,000rpm in JA17 (4℃)
10. Load the lysate onto a 10ml glutathione-Sepharose FF resin (Amersham Biosciences) pre-equilibrated in cold 1X PBS.
11. The settled column is washed with 100ml PBS.
12. Elute GST-MS2 with 40ml elution buffer *(for maximal yield, the first 30ml of eluate is collected immediately, and then the final 10ml of buffer is allowed to incubate on the column overnight at RT before collection. This final overnight elution contribute s approximately 25%of the final protein yield)*.
13. Fractions containing purified GST-MS2 are then dialyzed against three 1L changes of storage buffer. (12-14k cutoff, dialyse o/n) and store at -20℃
    - *The typical yield of purified GST-MS2 from 2L of culture is 50-100mg.*

Buffer and reagents for MS2 purification:

Stock solutions:

1M Tris-HCl, pH7.5 or pH8.0
3M KCl
1M DTT

Lysis buffer (50ml)	**Binding Buffer** (1L)	**Elution buffer** (100ml)	**Dialysis buffer** (3x1L)
20mM Tris-HCl, pH7.5 (1ml)	1x PBS (10x,100ml)	50mM Tris-HCl, pH8.0 (5ml)	1XPBS (10x,100ml)
150mM KCl (2.5ml)	2mM DTT (2ml)	10mM reduced glutathione 0.4g	50% glycerol (500ml)
2mM DTT (0.1ml)	H_2O ~900ml	(FW=307.33)	H_2O: 400ml
5% Glycerol (2.5ml)		H_2O ~ 95ml	
H_2O (43.9ml)			

10x PBS (pH7.4)

	10X(g)	1X(g)
NaCl	80	8 (137mM)
KCl	2	0.2 (2.7mM)
Na_2HPO_4	14.4	1.44 (10mM)
KH_2PO_4	2.4	0.24 (2mM)
H_2O	To 1L (pH7.4)	To 1L (pH7.4)

Affinity Column Purification of MS2-GST

Glutathion Sepharose 4 fast flow (amersham, cat# 17-5132-01)

Column size: 10ml
Store at 20% EtOH
Chart recorder: set sensitivity to 2.0
Turn UV lamp on (280nm)

CLEAN

Wash out EtOH with 3x column volumes ddH_2O, Flow rate 2ml/min

EQUILIBRATE

Wash with 3x column volumes binding buffer. Flow rate 2ml/min

LOAD

Remove any buffer above the column with pipette. Flow rate: 0.5ml/min

WASH

Wash the column with 10 column volumes of binding buffer. Flow rate: 2ml/min
Column should go from yellow to white.

ELUTE

Take any fluid above the column off, as this will ensure a rapid elution. Flow rate 0.75ml/min
If need, concentrate to ~5ml with concentrator (30K cutoff)

REGENERATE

Continue wash with elution buffer. Flow rate: 2ml/min
Wash the column with ddH_2O and leave o/n
Wash with 2 column volumes 6M Guanidine hydrochloride.
Wash with 5 column volumes ddH_2O.
Wash with 2 column volumes 20% EtOH,
Store at 4C.

Polyacrylamide gel analysis (10%)

Whole cell lysate
Supernatant (cleared lysate)
Flowthough
Eluted protein
Dialysed protein