Affinity purification of antibody using GST-Fusion proteins crosslinked to glutathione columns.

PROTOCOL

GST and GST-fusion protein column preparation

1. 5ml GST (pGEX-2TK) and 100ml GST-Zta (pGEX-2TK-Zta) bacteria grown overnight in 2×TY with 100ug/mL ampicillin.

2. Put GST culture into 50ml 2×YT media (with 100ug/mL ampicillin) and put GST-Zta culture into 1000ml 2×YT media (with 100ug/mL ampicillin).

3. Let cultures grown 1 hour at 37°C (in rotary shaker - 240 rpm).

4. Add IPTG to each culture (25ul of 1M IPTG into 50ml GST culture and 500ul of 1M IPTG into 1000ml GST-Zta culture).

5. Incubate at 37°C for 3 hours (in shaker).

6. Spin down bacteria (5000rpm for 5min at 4°C). From now on, keep on ice (all buffers should be prechilled).

7. Resuspend bacteria in NETN (chilled) using 1/20 original culture volume (2.5ml for GST and 50ml for GST-Zta).

8. Distribute into Sarstedt tubes (5 ml in each).

9. Sonicate on ice with three brief (10sec) pulses (in between pulses, put on ice and sonicate other samples one time each).

10. Spin down sonicate 10,000rpm for 10min at 4°C and pool supernatants for each culture.

11. Add glutathione sepharose beads (1:1 suspension in NETN) into supernatant (800 ul beads for GST and 2 ml beads for GST-Zta). (Bead preparation: suspend beads in 50 volumes of NETN, spin down beads (30 sec at 1K), add 10 volumes of NETN, incubate overnight at 4°C on rocker, spin down beads, take off supernatant and add 1 bead volume of NETN - beads are now ready).

12. Wash beads 2× with 10ml 0.1M borate buffer pH8.

13. Incubate beads in 10ml 40mM dimethylpimelimidate(0.12gm in 20ml 0.2M borate buffer pH9.0) on rocker for 1 hour in cold room.

14. Wash beads 2× with 10ml 0.1M borate buffer pH8.0.

15. Incubate beads with 10ml 40mM ethanolamine in 0.1M borate buffer pH 8 (24.4ul ethanolamine in 10ml 0.1M borate buffer on rocker for 45 min in cold room.
20 Wash 3× with 10ml cold PBS.
21 Wash 1× with 10ml 0.2M glycine-HCl(pH2.5).
22 Wash 1× with 10ml 1M K₂HPO₄.
23 Wash 1× with 10ml 0.2M glycine-HCl(pH2.5).
24 Wash 1× with 10ml 1M K₂HPO₄.
25 Wash 2× with 10ml PBS.
26 Store in equal volume PBS with 0.1% NaN₃ at -80°C or 4°C.

**Affinity purification of Antibody**

27 Wash GST column 1× with 10ml PBS (containing 0.2% Tween20) after removing storage buffer.
28 Dilute 4ml of serum (e.g. N.5) with 4ml with PBS (containing 0.2% Tween20), then rock with GST beads for 2 hours in cold room.
29 Spin down beads (2000 rpm, 2-5 sec) and save supernatant.
30 Wash beads with 1ml PBS (containing 0.2% Tween20); spin down beads; save supernatant and add to sup in 27.
31 (Can regenerate GST column with sequential glycine/K₂HPO₄ washes followed by PBS washes; store in equal PBS with 0.1% NaN₃).
32 After removing storage buffer of GST-Zta, wash it 1× with 10ml PBS (containing 0.2% Tween20).
33 Incubate supernatant from steps 29 and 30 with GST-Zta column overnight in cold room.
34 Place beads in BiaRad column (Poly-Prep chromatography column, Cat. 731-1550).
35 Pass 10ml PBS (containing 0.2% Tween20) through the column. Repeat 2×.
36 Pass 10ml PBS through column. Repeat 1×.
37 Elute Ab with 0.2M glycine-HCl(pH2.5). Add 750ul glycine-HCl(pH2.5) to column and collect 750ul eluate into an eppendorf tube containing 250ul 1M K₂HPO₄.
Repeat this process 9 times, so that 10 total fractions are collected.
38 Do protein determinations using the Biorad Protein Assay (Cat. 500-0006) (use 20ul of each fraction for protein determination).
39 Pool peak fractions (this has always been fractions 1-3 for our experiments).
40 Regenerate the GST-Zta column after use by washing the column 4× with 40ml of 1M Tris-HCl, pH8. Store column in PBS containing 0.1% NaN₃ at 4°C.
41 Dialyze (using dialysis tubing with MW 12-14k for proteins 20kdal or greater) overnight against 1000ml PBS/glycerol (PBS: glycerol is 1:1, mix and chill in ice water prior to use).
42 Add BSA to 1%. Store in 100ul fractions at -80°C.
43 Test antibody by western blot analysis.
SOLUTIONS

BORATE BUFFERS
1. **1M borate buffer (pH8.0)**
   100ml: 6.183g boric acid + 70ml ddH$_2$O. Add 10N NaOH to dissolve it then add more 10N NaOH to pH8.0. Add ddH$_2$O to bring volume up to 100ml.

2. **1M borate buffer (pH9.0)**
   Same as above, but pH9.0

3. **0.1M borate buffer (pH8.0)**
   400ml: Mix 40ml 1M borate buffer (pH8.0) plus 360ml ddH$_2$O.

4. **0.1M borate buffer (pH9.0)**
   100ml: Mix 10ml 1M borate buffer (pH9.0) plus 90ml ddH$_2$O.

5. **0.2M borate buffer (pH9.0)**
   100ml: Mix 20ml 1M borate buffer (pH9.0) plus 80ml ddH$_2$O.

OTHER BUFFERS
5. **1M K$_2$HPO$_4$**
   17.418g K$_2$HPO$_4$ in 100ml ddH$_2$O.

2. **1M glycine-HCl (pH2.5)**
   200ml: 15.01g glycine-HCl in 200ml ddH$_2$O, adjust pH with HCl to pH2.5.

6. **0.2M glycine-HCl (pH2.5)**
   100ml: 20ml 1M glycine-HCl (pH2.5) plus 80ml ddH$_2$O.

4. **40mM ethanolamine**
   48.8ul stock solution (Sigma#A7177) in 20ml 0.1M borate buffer (pH8.0).

5. **40mM Dimethylpimelimidate**
   0.12g Dimethylpimelimidate (Sigma#D8388) in 20ml 0.2M borate buffer (pH9.0). Adjust pH to 9.0 using 10N NaOH.

6. **NETN**
   0.5% NP-40(Sigma#N0137).........................NP-40 2.5ml
   20mM Tris (pH8.0)........................................1M Tris 10ml
   100mM NaCl.............................................5M NaCl 10ml
   1mM EDTA..............................................0.5M EDTA 1ml
   add ddH$_2$O to 500ml