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-Zta②) 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 3 with 10ml NETN (at least 10 bead volumes).
13 Centrifuge 2000 rpm for 3sec.
14 Wash beads 2× with 10ml 0.1M borate buffer pH8.
15 Wash beads 1× with 10ml 0.1M borate buffer pH9.
16 Wash beads 1× with 10ml 0.2M borate buffer pH9.
17 Incubate beads in 10ml 40mM dimethypimelimidate(0.12gm in 20ml 0.2M borate buffer pH9.0) on rocker for 1 hour in cold room.
18 Wash beads 2× with 10ml 0.1M borate buffer pH8.0.
19 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 K_2HPO4.
23 Wash 1× with 10ml 0.2M glycine-HCl(pH2.5).
24 Wash 1× with 10ml 1M K_2HPO4.
25 Wash 2× with 10ml PBS.
26 Store in equal volume PBS with 0.1% NaN_3 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 sequental glycine/K_2HPO4 washes followed by PBS washes; store in equal PBS with 0.1% NaN_3).
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 K_2HPO4. Repeat this process 9 times, so that 10 total fractions are collected.
38 Do protein determinations using the Biorad Proetin 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% NaN_3 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_2O. Add 10N NaOH to dissolve it then add more 10N NaOH to pH8.0. Add ddH_2O 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_2O.
4. 0.1M borate buffer(pH9.0)
   100ml: Mix 10ml 1M borate buffer (pH9.0) plus 90ml ddH_2O.
5. 0.2M borate buffer(pH9.0)
   100ml: Mix 20ml 1M borate buffer (pH9.0) plus 80ml ddH_2O.

OTHER BUFFERS

5. 1M K_2HPO_4
   17.418g K_2HPO4 in 100ml ddH_2O.
2. 1M glycine-HCl (pH2.5)
   200ml: 15.01g glycine-HCl in 200ml ddH_2O, adjust pH with HCl to pH2.5.
6. 0.2M glycine-HCl (pH2.5)
   100ml: 20ml 1M glycine-HCl (pH2.5) plus 80ml ddH_2O.
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_2O to 500ml	