Peptide Antibody Production

A) Peptide

BioSynthesis (http://www.biosyn.com, 800-227-0627)

B) Conjugation of peptide to KLH

(Imject Maleimide Activated KLH, PIERCE=Thermo #77605, 10 mg)

C) Peptide affinity column

(SulfoLink coupling gel, PIERCE=Thermo #20401)

D) Immunization

E) Purification of antibody

A) Peptide

At least 20 mg peptide is required. Order 20-40 mg scale, >75% pure synthesis at BioSynthesis (http://www.biosyn.com, 800-227-0627).

Make 24-25 aa peptide and add Cys to COOH (or NH4) terminal for conjugation (final length of the peptide is 25-26 aa).

B) Peptid-KLH Conjugation and C) Peptide Affinity Column

Note: If there is enough peptide, make two affinity columns for each rabbit.

1. Dissolve 20 mg peptide to 400 ul dH2O (50 mg peptide /ml dH2O) just before conjugation. Make aliquote of 180 ul (for KLH conjugation), 180 ul (for Affinity colomn) and the rest (40ul).

Note: If peptide is insoluble, dissolve in a tiny volume of DMSO, and then add dH2O. DMSO has to be <30% in final conjugation solution or carrier protein may irreversibly denature.

For Conjugation/immunization:

B. 2. Dissolve 10 mg IMA-KLH to 1 ml dH2O (10 mg/ml), use 500 ul (the rest can be kept frozen at -80C for a few days).

B. 3. Add 20 ul 10X PBS to 180 ul of peptide soln (containing 9 mg peptide).

B. 4. Immediately mix 500 ul IMA-KLH (10 mg/ml) and peptide (50 mg/ml).

B. 5. Stir at RT for 2 hrs

For Affinity column

C. 2. Add 320 ul dH2O and 500 ul 10X TE (100 mM Tris pH8.0, 10 mM EDTA) to 180 ul of peptide (9 mg peptide) (from solution made in 1)

C. 3. Add mix to the (rinsed) SulfoLink (1.5 ml slurry into 15 ml tube, spin down and wash 4 times with 5X TE).

C. 4. Rotate vigorously for 90 min at RT.

Note: Add 360 ul PBS to the rest of peptide (40 ul) and store at -80C.

Then for immunization:

B. 6. Dialyse against PBS O/N. After dialysis, bring up to 2 ml final vol. with PBS.

B. 7. Consider coupling efficiency at 20%, so this ends up to 2 mg/ml coupled peptide (i.e. 2 mg peptide + 5mg KLH in 2 ml PBS).

B. 8. Aliquot for immunization of 2 rabbits:

Tube#1 200 ug peptide per rabbit = 400 ul peptide-KLH, add 600 ul PBS to final 1ml.

Tube#2-6 100 ug per rabbit = 200 ul pep-KLH, add 800 ul PBS to final 1 ml.

Freeze down the rest of peptide-KLH at -80C.

Then for affinity column

C. 5. Spin down the beads, remove the sup and save for later use.

C. 6. To block unused sites, add 1ml 5X TE and 15.6 ul of b-mercaptoethanol (100 mM final conc.). Rotate for 15 min at RT.

C. 7. Wash three times with 5X TE.

C. 8. Transfer to a column and wash with PBS until no b-ME smell remains.

C. 9. Store at 4°C in PBS/0.02 % sodium azide.

D) Immunization

Immunize 2 rabbits for each peptide at Covance (800-345-4114, http://www.covance.com).

Day0 NZW female, prebleed (5ml serum). 1st ID: Tube #1 with FCA (final 2ml for 2 rabbits).

Day21 Boost SC: Tube #2 with FIA.

Day42 Boost SC: Tube #3 with FIA.

Day52 Test bleed (5 ml serum)

Day63 Boost SC: Tube #4 with FIA.

Day73 Production bleed (20 ml serum).

Day84 Boost SC: Tube #5 with FIA.

Day94 Production bleed (20 ml serum).

Day105 Boost SC: Tube #6 with FIA.

Day115 Production bleed (20 ml serum).

Day118 Terminal bleed (50 ml serum)

E) Purification of antibody

1. The rabbit sera come from the company in a volume of 15-20 ml per bleed. Store them the way they come at -70°C until it is thawed for purification. The first time a bottle is opened, thaw it at RT or at 4°C overnight. Pass the whole content through a 0.45 um filter linked to a syringe. Divide the serum into 5ml aliquots in 15 ml Falcon tubes. Clearly label the date of the bleed, use 5-10 ml per purification and store the rest at -70°C.

2. (optional) Spin crude serum for 10 min 4°C at 15K rpm to remove debris.

3. Wash the affinity column with 30 ml PBS.

4. In cold room, apply 5-10 ml serum to 1 ml packed resin in the column. Pass serum through column 4 more times.

5. Wash column with

   40ml PBS

   40 ml PBS/0.5 M NaCl

   40 ml PBS

6. Elute in 8 ml 0.1 M glycine pH 2.5.

7. Collect 1 ml fractions (label them as #1-8) into 65 ul 1M Tris pH 9.5. Check the final pH is around 7 (by pipetting a ul on a pH paper strip).

8. Assay 20 ul samples using Bradford protein assay (BioRad #500-0006, 20 ul sample, 1 ml of 1:5 dilution of assay reagent, no need to set up BSA standards). The peak should be in the first few fractions.

9. Combine all fractions with OD595>0.1 (usually the first 3-4 fractions).

10. Dialyse it O/N against PBS / 20 % glycerol in cold room.

11. After dialysis, there may be some precipitate. Transfer the whole content (including the precipitate) to a 15 ml falcon tube. Do not attempt to separate the precipitate by centrifugation, because it would lead to excessive antibody loss. Much of the precipitate will slowly dissolve in storage.

12. Measure the antibody concentration using Bradford assay against BSA standards. The antibody concentration is usually from 0.1 to over 1 mg/ml. If the antibody is very diluted (e.g. <0.1 mg/ml), add BSA to 1 mg/ml for stabilization.

13. Add 0.02 % sodium azide (final conc.), aliquot, and store at -70 °C. Keep using stock at 4°C.

14. Wash the column with 20 ml of PBS / 0.02 % azide and store at 4 °C sealed.