Testing Rabbit Bleeds By Elisa

Claire Walczak January 1996

You can test whether or not you have gotten an immune response to the peptide and how strong that immune response is by doing ELISAs against peptide conjugated to BSA. By conjugating to BSA, you will eliminate any signal for antibodies generated to KLH during immunization.

Protocol:

A. Coupling Peptide to BSA

You need:

- 1 ml of 2 mg/ml BSA in 0.1 M $NaHCO_3$.
- 1 ml of 0.2% glutaraldehyde in 0.1 M $NaHCO_3$.
- 0.5 ml peptide (1 mg/ml in DMSO). Peptide can be added as a solid if soluble.

1. Mix, adding glutaraldehyde last. Peptide and BSA turn a little yellow even before adding glutaraldehyde.
2. Incubate 90' at 37 deg C.
3. Add 0.1 volumes of 0.1 M $NaBH_4$ in 0.1 M $NaHCO_3$. There will be some bubbling. Add same amount of $NaBH_4$ after 15'. Do a quick microfuge spin if there are too many bubbles.

B. ELISA with Peptide conjugated to BSA

1. Coat 10 ug/ml antigen (peptide conjugated to BSA) diluted in TBS (50 ul/well) ON at 4 deg C.
2. Remove antigen and rinse wells 2Xs with TBST.
3. Block 2 hr with 200 ul of 5% NFDM in TBST.

4. Remove blocking reagent and rinse wells 2Xs with TBST.
5. Incubate in primary antibody diluted in Blocking Buffer for 2 hr at RT. I do tripling dilutions beginning at 1/10 (50 ul/well).
6. Remove primary antibody and rinse wells 4Xs with TBST.
7. Incubate in secondary antibody (1/5000 Goat anti-rabbit conjugated to AP) diluted in Blocking Buffer for 1 hr at RT (50 ul/well).
8. Remove secondary antibody and rinse wells 4Xs with TBST.
9. Rinse wells 2Xs with 50 mM HCO_3; 0.5 mM $MgCl_2$, pH 10.
10. Develop in 1 mg/ml p-Nitrophenyl phosphate in 50 mM HCO_3; 0.5 mM $MgCl_2$, pH 10 (50 ul/well).
11. Read A410 in ELISA reader.