



2 and 3 Peptide Cocktail Projects

Peptide antibodies do not always recognize the native protein, due in large part to the differences in tertiary structure between a synthetic peptide of 10-35 amino acids and a folded protein. One approach to increase the probability that a peptide project will work for multiple applications and recognize the native protein is to inject two or more peptides against the same protein into one set of animals. The reason for the increase in applicability is two-fold: 1) the larger numbers of amino acids from the individual protein provide additional epitopes for antibody response, particularly in immuno-precipitation. 2) each antibody can be characterized separately, from multiple animals; since each animal responds differently to an antigen the researcher now obtains four or six antibodies to their protein of interest against multiple sites, at minimal additional cost. However, the process of producing antibodies against peptide cocktails is a bit more complicated than traditional antibody production.

Step 1: Synthesize two peptides

- Peptide #1: peptide sequence of interest

- Peptide #2: other peptide sequence of interest


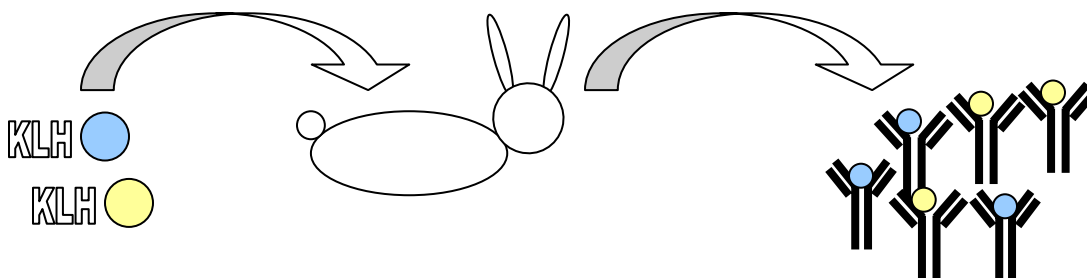
Step 2: Conjugate Peptide

- Conjugate each peptide to a carrier protein, such as KLH



Step 3: Immunization protocol

- Immunize both peptide conjugates into animal(s)
- Take production bleeds to obtain serum at scheduled intervals



Step 4: Evaluate serum

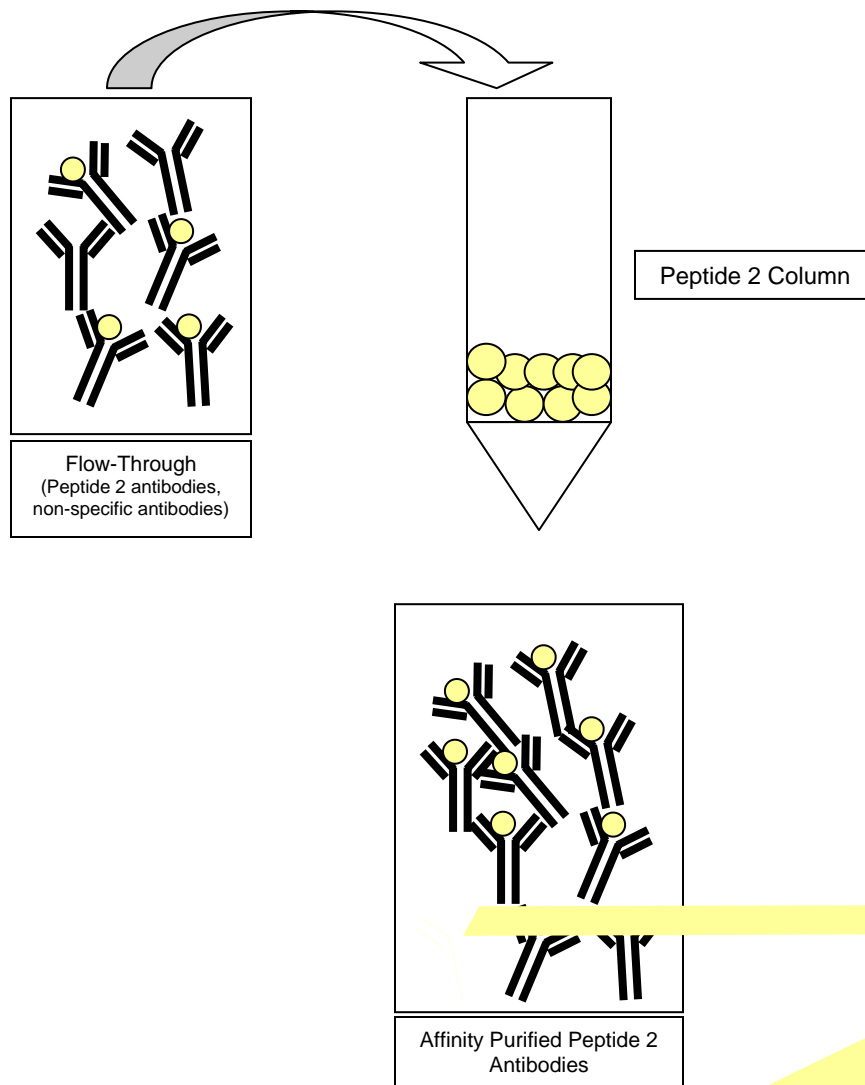
- Serum contains a mixture of antibodies; some of the antibodies will be against each of the peptides

Whole Serum
contains a mixture of
antibodies against
each peptide

Step 5: Purify Serum

- Separating the antibodies from each peptide is performed by dual immuno-affinity purification, using two columns, one specific to each peptide:
 - Peptide 1 column: bind Peptide 1 to gel matrix for purification
 - Peptide 2 column: bind Peptide 2 to gel matrix for purification

- Run the whole serum through the Peptide 1 column. The eluted affinity purified antibody will contain antibodies specific to Peptide 1. The flow-through will contain a mixture of antibodies against Peptide 2 as well as other antibodies present in the serum
- Run the flow-through from the Peptide 1 column through the Peptide 2 column. The eluted affinity purified antibody from the Peptide 2 column will contain only the Peptide 2 antibody



- Run ELISAs on the whole serum, the affinity purified antibody from the Peptide 1 column and Peptide 2 column, and the flow-through from the Peptide 1 column and Peptide 2 column to determine titers. Run flow-through from the Peptide 2 column to determine if additional antibody is still present.