



## Projects with Phosphorylated Residues

One of the most common peptide-modifications used for antibody production is the phosphorylation of residues; this can ensure that the peptide mimics the natural conformation of the protein. Phosphorylation often results in functional changes in the protein and having antibodies recognizing the phosphorylated state can be very advantageous for studying protein pathways. However, the process of producing antibodies against a phospho-residue peptide a bit more complicated than traditional antibody production.

There are three residues which can be phosphorylated:

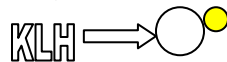
- Serine (S)
- Threonine (T)
- Tyrosine (Y)

### Step 1: Synthesize two peptides

- Peptide #1 (non-phospho peptide): the peptide sequence of interest without the added phosphorylation on the residue of interest  

- Peptide #2 (phospho peptide): the peptide sequence of interest with the phosphorylation. The ideal peptide sequence for phospho production is to have a shorter sequence to minimize production against the non-phospho regions of the sequence and to highlight the phosphorylated residue by centering it:  
 C-XXXXX(pX)XXXXX where C is added for conjugation and X is any residue.  


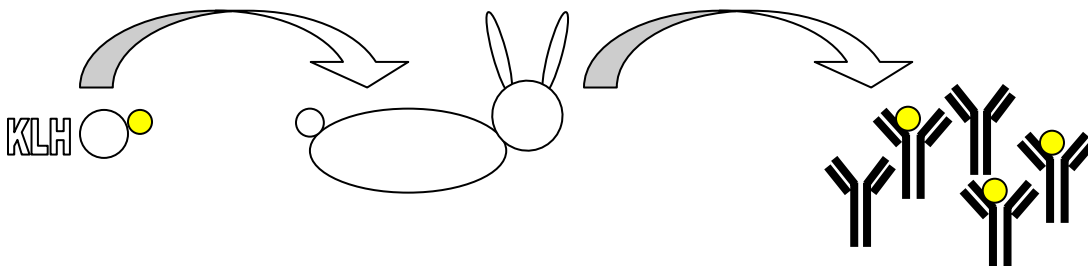
### Step 2: Conjugate Peptide

- Conjugate the phospho-peptide to carrier protein, such as KLH



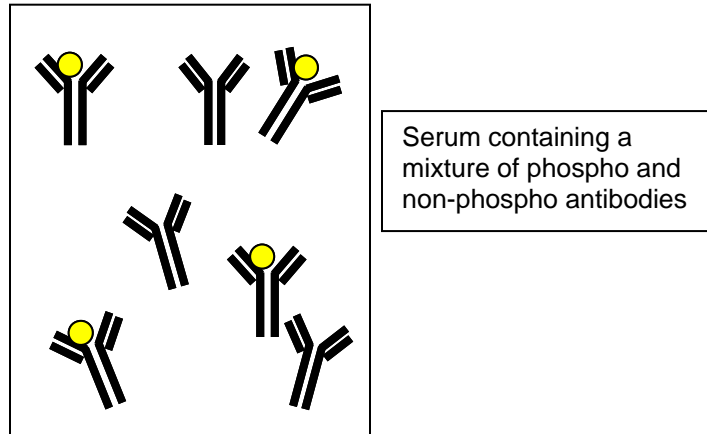
### Step 3: Immunization protocol

- Immunize phospho-peptide conjugate into animal(s) at scheduled intervals
- Remove serum at scheduled interval



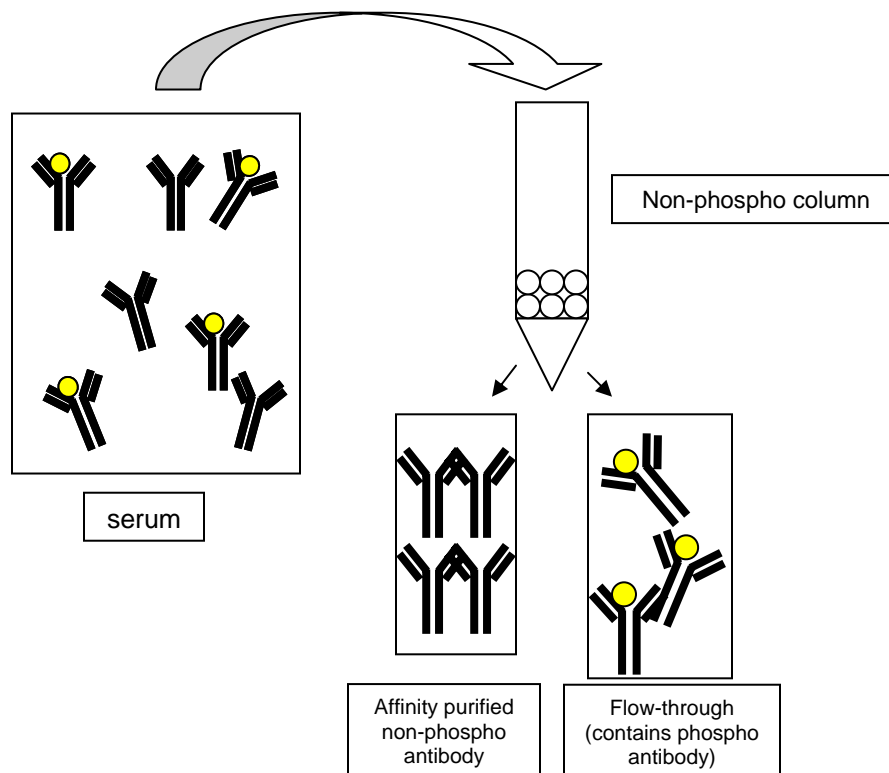
#### Step 4: Evaluate serum

- Serum contains a mixture of antibodies; some of the antibodies recognize the phosphorylated residue, while some antibodies will not.



#### Step 5: Purify Serum

- Separating the phosphorylated antibodies from the non-phosphorylated antibodies is performed by immuno-affinity purification, using two columns:
  - Non-phospho peptide column: bind the non-phospho peptide to gel matrix for purification
  - Phospho column: bind the phospho peptide to gel matrix for column



- Run the serum through the non-phospho column. The eluted affinity purified antibody will contain antibodies without the phosphorylated residue. The flow-through will contain a mixture of phospho antibodies as well as other antibodies present in the serum
- Run the flow-through through the phospho column. The eluted affinity purified antibody will contain the specific, phosphorylated antibody
- Use the phospho peptide to run an ELISA on the serum, phospho affinity purified antibody, and the flow-through resulting from the phospho column

