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Validation of Humanized Mouse Antibodies

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Validation of Humanized Mouse Antibodies MeiLing Norfolk & Rocco Rotello, Ph.D. School of Pharmacy, Cedarville

Introduction:

- Mouse monoclonal antibodies can be "humanized," or cloned until the heavy and light chains of the antibody are no longer recognized as "self" by human cells.
- Assays ensure the antibodies are still functional and retain specific binding to human cell targets.
- Successful antibodies may go on to clinical trials for antibody therapies (ex. Lucentis® for macular edema)



Methods:

- 1) Purification of mouse IgG1
 - harvest cell supernatant
 - clarify
 - affinity chromatography



- 2) ELISA for antibody binding
 - specific binding of antibody to target protein, correct
 - anformation structure
 - conformation, structure
 - protein tested is human beta
 - cross-reactivity tested with cynomolgus beta protein
 - negative control is human eta protein



 3) Western blot for size determination

 shows specific binding of denatured protein to antibody
 same experimental proteins as ELISA



Results:

- Lead monoclonal antibody retained specificity in binding and functionality
- Native and denatured proteins are recognized by lead antibody
- Target and antibody matched



Future Research:

- Testing the antibody products for safety in animal models
- Testing the antibody products for use in humans