

September 30th, 2009
Biochemistry 660

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Attie Lab

Outline

Overview of Antibodies

Structure

Classes

Modifications

Immunological Techniques

Western Blotting

Immunoprecipitation

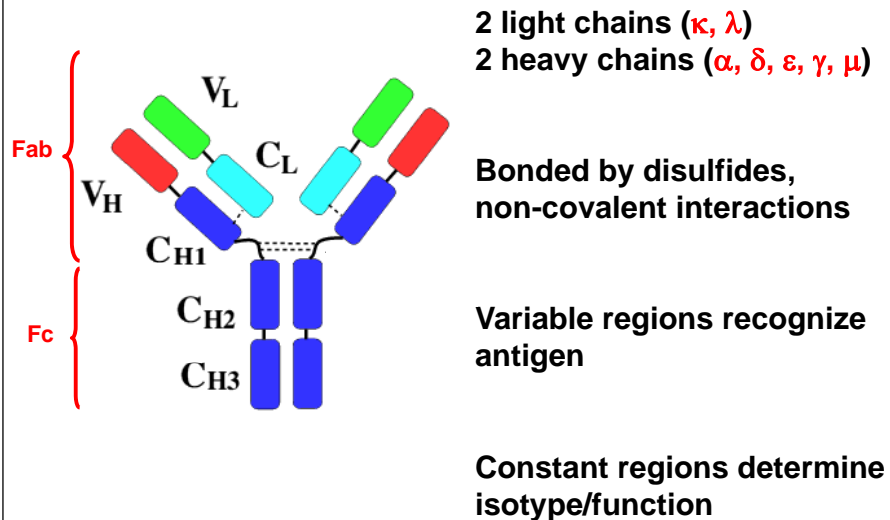
Immunofluorescence

Immunocytochemistry, Immunohistochemistry

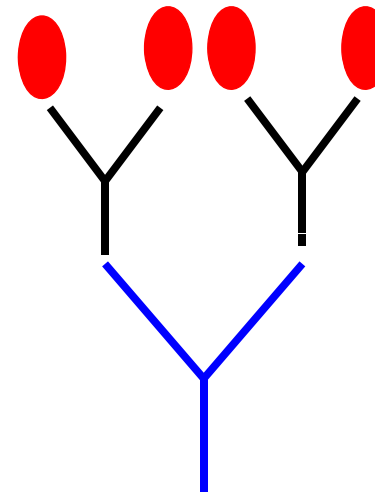
Enzyme-Linked ImmunoSorbant Assay

Radioimmunoassay

Antibody Structure



Technical Terminology



Antigen

"Insulin"

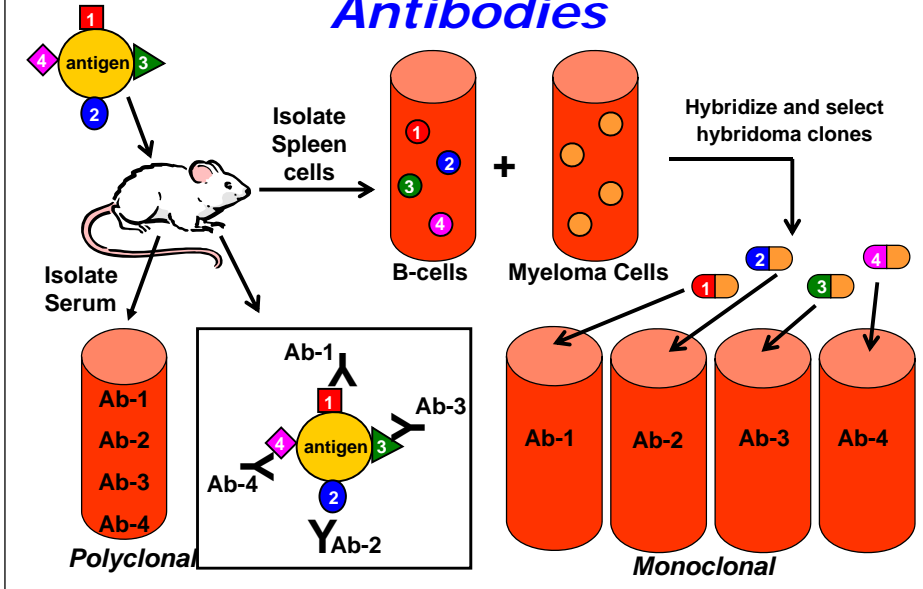
1° Antibody

"Mouse anti-insulin IgG"

2° Antibody

"Goat anti-mouse IgG"

Polyclonal vs. Monoclonal Antibodies



Polyclonal vs. Monoclonal Antibody Pros and Cons

Polyclonals

Resistant to epitope confirmation
 Inexpensive
 Relatively rapid
 Technically easy

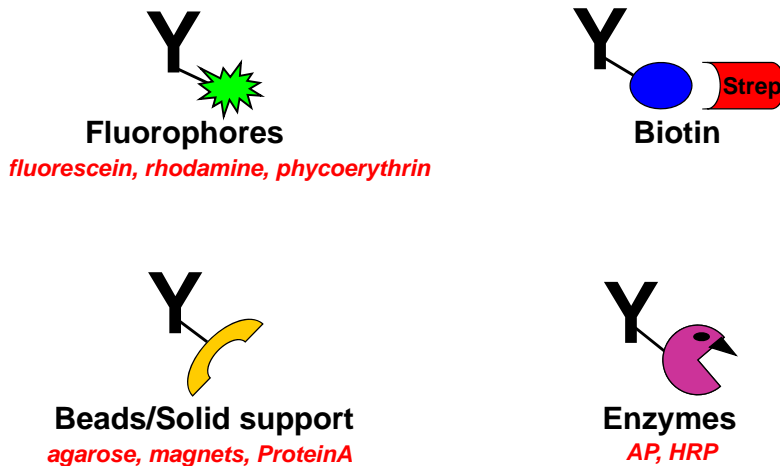
Low reproducibility
 Cross-Reactive
 Limited supply

Monoclonals

Highly reproducible
 Specific
 Limitless supply

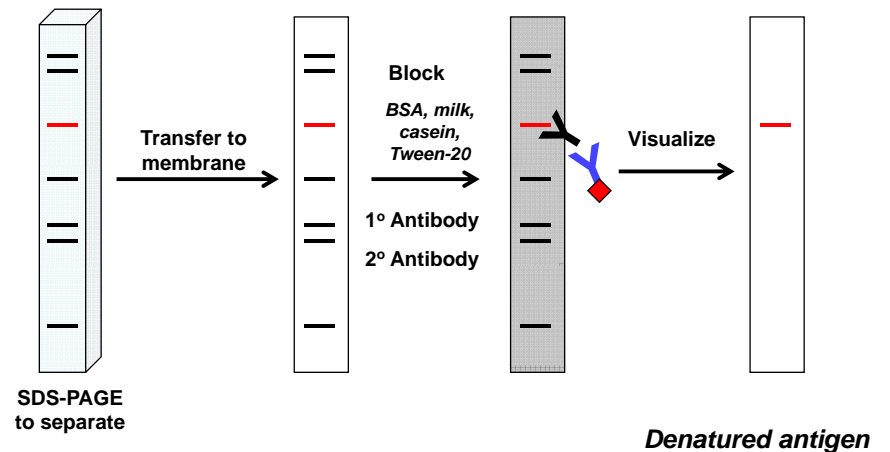
Sensitive to epitope conformation
 Expensive
 Time-consuming
 Technically difficult

Antibody Modifications

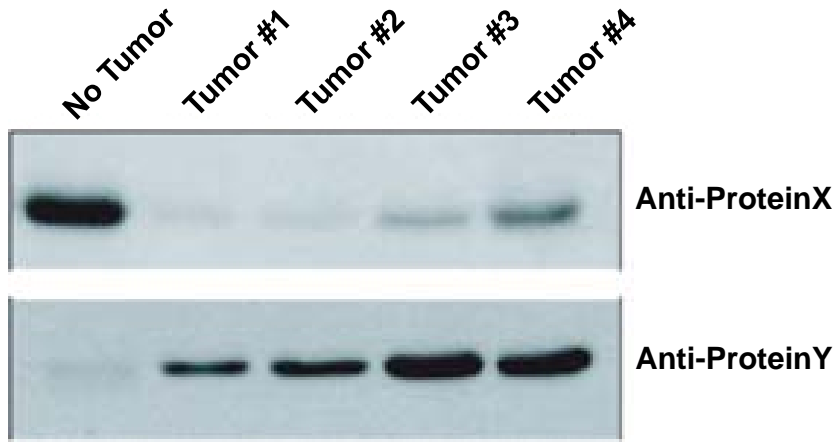


Western Blotting

1D method to identify a specific protein from a complex mixture



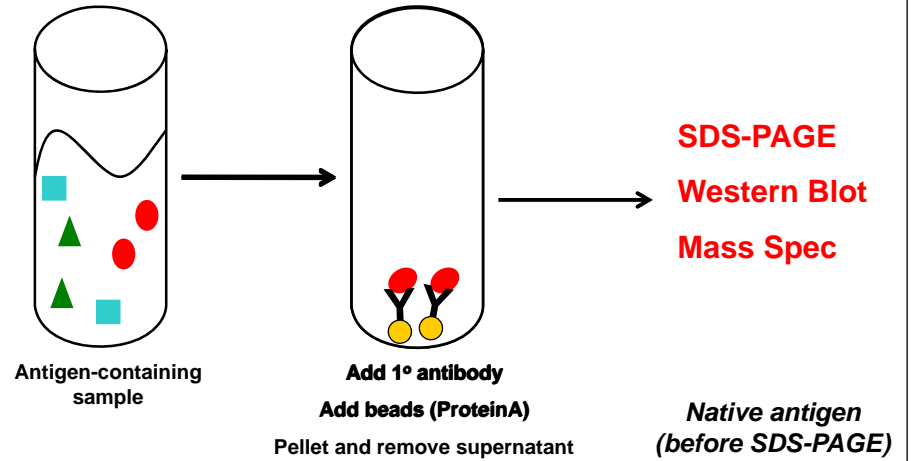
Western Blotting



Adapted from Christofk et al. (2008) Nature 452: 230-234

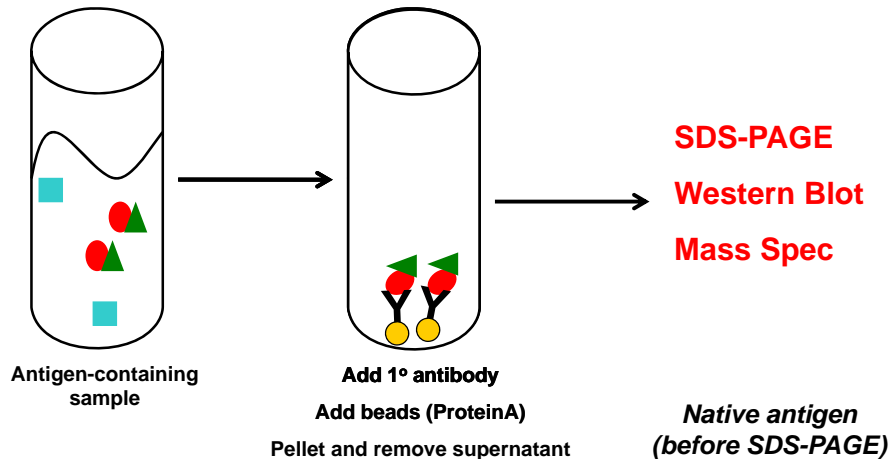
Immunoprecipitation

Isolate antigen in solution for further analysis

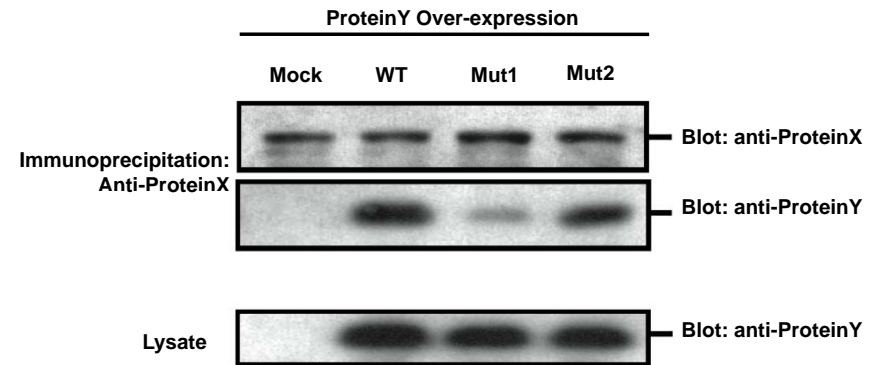


Co-immunoprecipitation

Isolate antigen complex in solution for further analysis



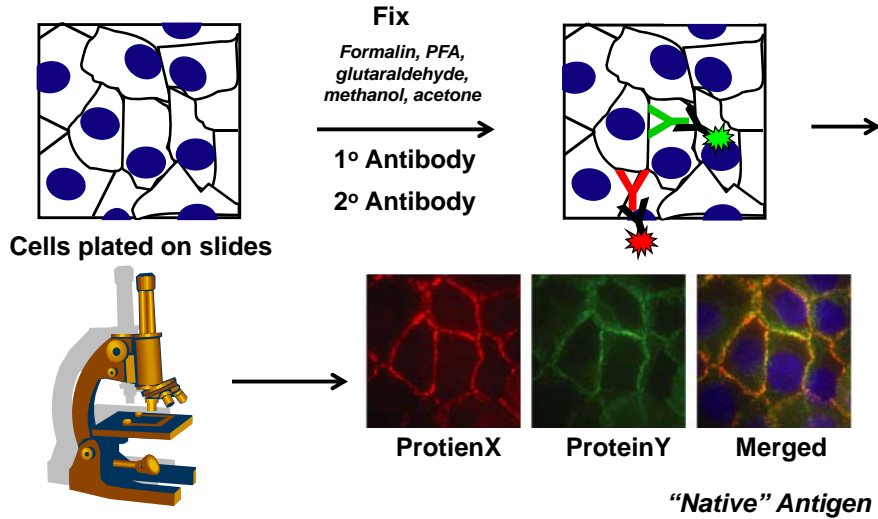
Co-immunoprecipitation



Adapted from Bennasser and Jeang. (2006) Retrovirology 3:95

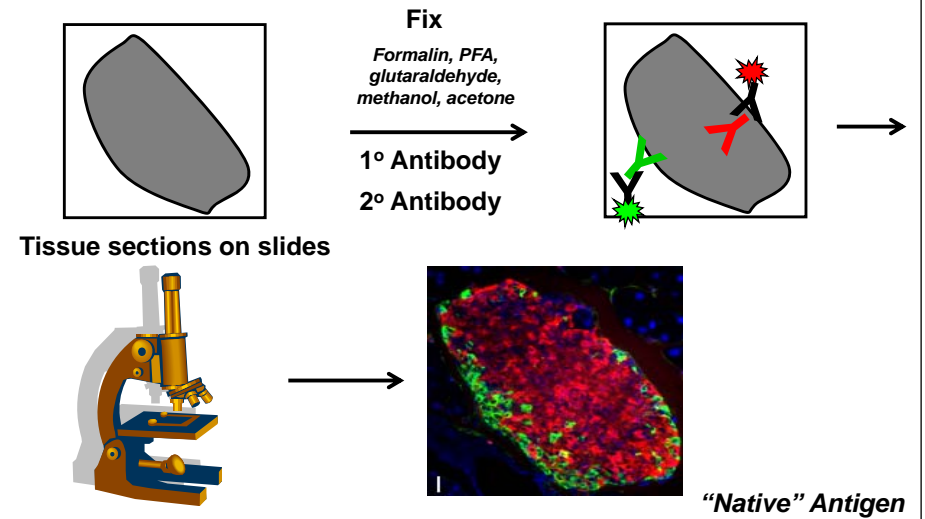
Immunocytochemistry

Image antigen in live or fixed cells

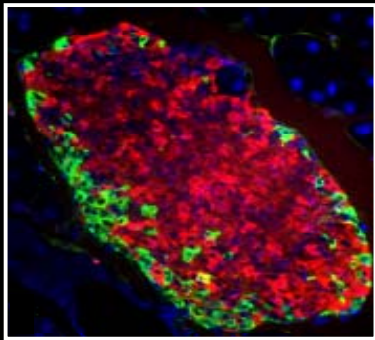


Immunohistochemistry

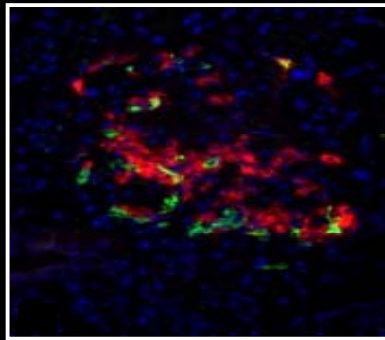
Image antigen in fixed tissue



Immunohistochemistry



Non-diabetic



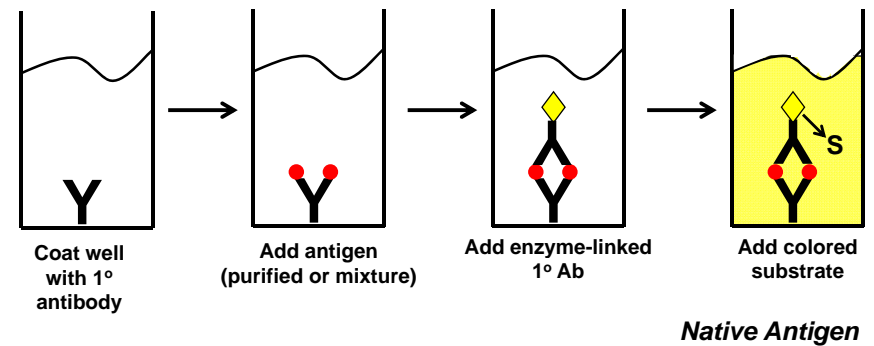
Diabetic

Nuclei (DAPI)
 β -cells (Protein X)
 α -cells (Protein Y)

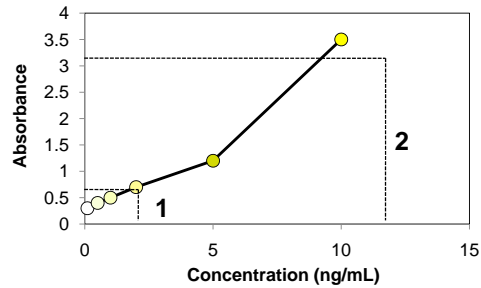
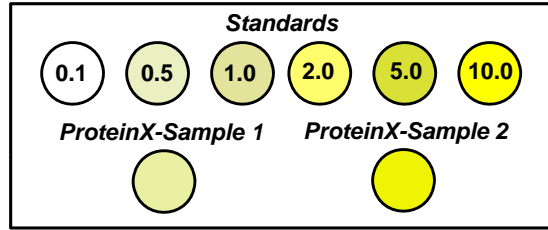
Enzyme-Linked ImmunoSorbant Assay (ELISA)

Quantitative detection of antigen or antibody from a complex mixture

Sandwich Method

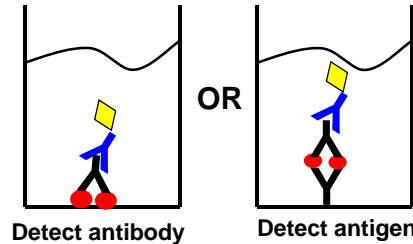


ELISA

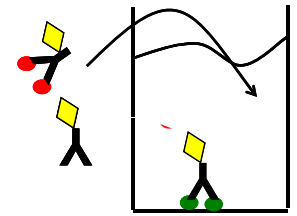


Variations on ELISA

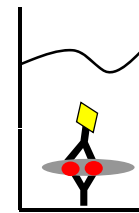
Indirect Method



Competitive Method



ELISPOT Method

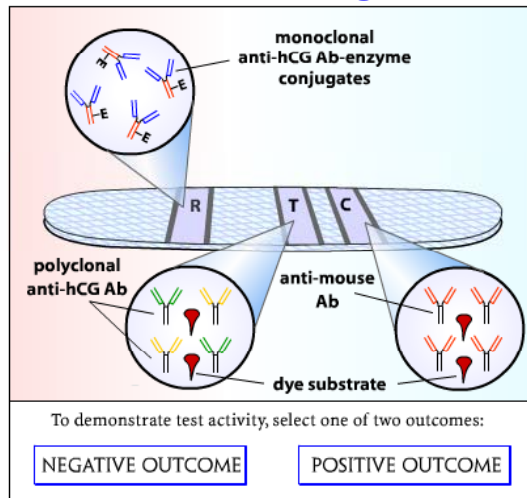


Detect cells expressing or secreting antigen

React antigen1 with labeled antibody before adding to antigen2-labeled well

less color = better binding to antigen1

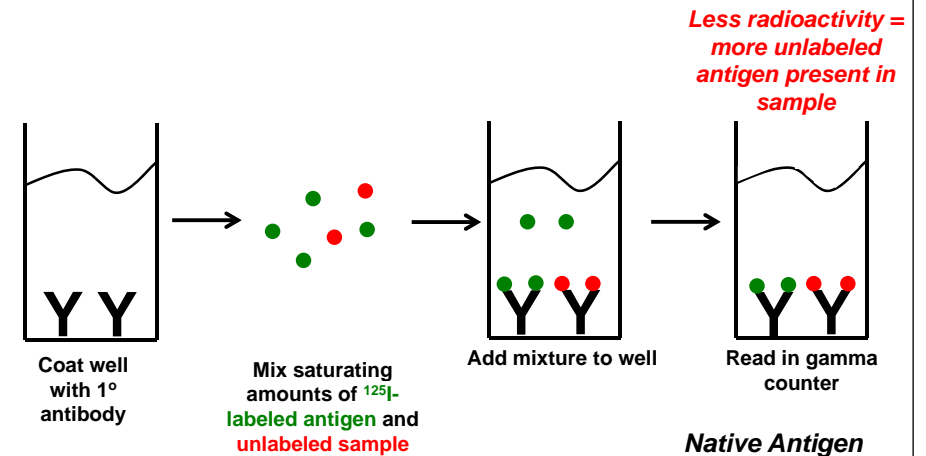
An ELISA Example – Are You Pregnant?



http://www.whfreeman.com/kuby/con_index.htm?06

Radioimmunoassay (RIA)

Quantitative detection of antigen from a complex mixture



Common Questions Asked

	<u>Western</u>	<u>IP</u>	<u>IF</u>	<u>ELISA</u>	<u>RIA</u>
Protein Expression?	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Protein up- or down- regulation?	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Sub-cellular localization?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Protein binding partners?	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Post-translational modification?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Protein/Metabolic profile?	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Disease diagnostics? <i>Antibodies against foreign proteins?</i>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>

Summary of Techniques

	Excellent Pot.	Very good	Good	Fair
	<u>Easy</u>	<u>Cheap</u>	<u>High through-put</u>	<u>Quantitative</u>
Western	●	●	○	◐
Immunoprecipitation	◐	○	●	◐
Immunofluorescence	●	◐	●	○
ELISA	◐	◐	◐	●
Radioimmunoassay	○	●	◐	●

Antibody Sources

Commercial

Molecular Probes (<http://probes.invitrogen.com>)

Jackson Labs (www.jacksonimmuno.com)

BD Pharmigen (wwwbdbiosciences.com)

Abcam (www.abcam.com)

Services

Hybridoma/monoclonal production

Custom-made polyclonals

