

Immunocytochemistry
Tissue sectioning
&
Transcardial Perfusion

Immunostaining

- Detect tissue/cellular distribution of protein

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

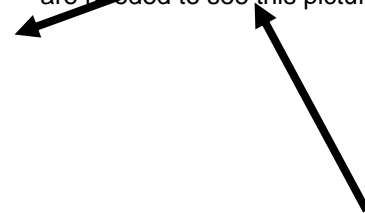
Procedure

- Tissue fixation
 - Transcardial perfusion
 - Paraformaldehyde in PBS



- Wear protective
(eye glass/glove)

QuickTime™ and a
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are needed to see this picture.



Tissue sectioning

- Freezing Microtome
 - Cut fixed tissue to 40-50 μm
 - Freeze tissue before and while sectioning

Polyclonal Antibody

- Antigen - purified protein/partial peptide
- Inject to various animals (other than mouse)
 - Lab level: rabbit
 - Commercial production: sheep, goat, horse
- Check titer by western or ELISA

Monoclonal Antibody

- Monoclonal (mouse)

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Detection methods

- Requires high spatial resolution
 - HRP and DAB
 - Gold particle (electron microscopy)
 - Fluorescence (multiple labeling)
 - Chemiluminescence is not appropriate. Why?

Fluorescence detection

- Anti-GFAP (rabbit) < anti-rabbit IgG (goat)-fluorescein (green)
- Anti-NeuN (mouse) < anti-mouse IgG (goat)-Texas-Red (red)

This does not work- why?

- Anti-GFAP (goat) < anti-goat IgG (horse)-fluorescein (green)
- Anti-NeuN (mouse) < anti-mouse IgG (goat)-Texas-Red (red)

This also does not work- why?

- Anti-GFAP (goat) < anti-goat IgG (horse)-fluorescein (green)
- Anti-NeuN (mouse) < anti-mouse IgG (sheep)-Texas-Red (red)

Outline

- Start primary antibody reaction
- 1 h incubation
- (Transcardial perfusion)
- Wash and second antibody reaction
- 1 h incubation
- (Tissue sectioning)
- Wash and observation