Immunocytochemistry
Tissue sectioning
&
Transcardial Perfusion
Immunostaining

• Detect tissue/cellular distribution of protein
Procedure

• Tissue fixation
  – Transcardial perfusion
  – Paraformaldehyde in PBS

  – Wear protective (eye glass/glove)
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 ELIZA
Monoclonal Antibody

• Monoclonal (mouse)
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