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# Staining retinal cryostat sections

*PBS instead of Sorenson's can be used

**Acronyms and shorthands:**

| | |
|---|---|
| PFA | Paraformaldehyde |
| PBS | Phosphate-buffered saline |
| Sor | Sorenson´s buffer |
| NDS | Normal Donkey Serun |
| RT | Room temperature |
| O/N | Over night |

## Immunostaining

**Day1:**

1. Thaw slides for **30min** at **RT**
2. Have chamber with wet tissue paper ready
3. Draw a barrier with hydrophobic pen
4. Remove OCT/NEG50 by short incubation (**5min**) in PBS or Sorenson's (use slide mailer if many slides or chamber if only a few slides) at **RT**.
5. Incubate in **5% NDS with 0.5% Triton-X in PBS** (or Sorenson's) for **1h-2h** at **RT** in chamber.
6. Incubate in primary antibody in **5% NDS with 0.5% Triton-X in PBS** (or Sorenson's) **overnight** at **4°C** in chamber.

**Day2:**

7. Wash at least **5x 10min in PBS** (or Sorenson's), shaking in slide mailer
8. Incubate in secondary antibody for **1.5h** at **RT** in **5% NDS with 0.02% Triton-X in PBS** (or Sorenson's) in chamber. Cover with foil to protect from light from this point on.
9. **5x 10' wash in PBS** (or Sorenson's), shaking in slide mailer
10. Mount with DAPI Fluoromount