Contents

[Procedure 1 (Heading 1) 1](#_Toc467688079)

[Task 1 of Procedure 1 1](#_Toc467688080)

[Task 2 of Procedure 1 1](#_Toc467688081)

[References 2](#_Toc467688082)

# Buffers

### PTx.2 (1L)

* 100mL PBS 10X
* 2mL TritonX-100

### PTwH (1L)

* 100mL PBS 10X
* 2mL Tween-20
* 1mL of 10mg/mL Heparin stock solution

### Permeabilization Solution (500mL)

* 400mL PTx.2
* 11.5g of Glycine
* 100mL of DMSO

### Blocking Solution (50mL)

* 42mL PTx.2
* 3mL of Donkey Serum
* 5mL of DMSO

# Sample Collection

1. Anesthetize the mouse.

2. Perfuse with 10mL PBS.

3. Perfuse with 10mL 4%PFA/PBS.

4. Dissect the brain/organ and trim to the appropriate size.

5. Fix in 1xPBS/4%PFA at 4°C, 48hr with shaking.

6. Wash in PBS with shaking: RT 30min x 3times.

Add screen shots for clarity:

# Immunolabeling

After fixation and wash:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tissue | Dehydrate? | 5% H2O2 in EtOH1 |  |  |  |  |
| Brain | O/N | O/N |  |  |  |  |

1. Bleach in chilled fresh 5%H2O2 in methanol (1 volume 30% H2O2 to 5 volumes MeOH), overnight at 4°c.

# Clearing tissue

After immunolableing:

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Tissue | 50% EtOH + 2% Tween (pH 9) | 75% EtOH + 2% Tween (pH 9) | 2x 100% EtOH + 2% Tween (pH 9) | 2x ECi |  |  |
| Brain | 24hr | 24hr | 24hr each | 4hr each |  |  |

# Tissue check

How clear does it look? Yellowish?

# References

* Hyperlink or paper citation
* <https://idiscodotinfo.files.wordpress.com/2015/04/whole-mount-staining-bench-protocol-methanol-dec-2016.pdf>
* Klingberg et al., Fully Automated Evaluation of Total Glomerular Number and Capillary Tuft Size in Nephritic Kidneys Using Lightsheet Microscopy, 2017.