Sample type	Incubation time (n)	Solution volume
Embryonic:		
E10-E11 embryo	1d	1.6mL
E12 embryo	2d	1.6mL
E13-E14 embryo	3d	1.6mL
E15-16 embryo	4d	4mL
E18 head	4d	4mL
Adult organ	4d	1.6mL
Adult brain:		
hindbrain + cerebellum	3d	1.6mL
cut hemisphere	5d	1.6mL
Whole brain	7d	4.5mL

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

Immunolabeling

1. Incubate samples in **Permeabilization Solution**, 37°C $^{n}/_{2}$ days (max. 2 days)
2. Block in **Blocking Solution**, 37 °, $^{n}/_{2}$ days (max. 2 days).
3. Incubate with primary antibody in **PTwH**/5%DMSO/3% Donkey Serum, 37°, **n** days.
4. Wash in **PTwH** for 4-5 times until the next day.
5. Incubate with secondary antibody in **PTwH**/3% Donkey Serum, 37°, **n** days.
6. Wash in **PTwH** for 4-5 times until the next day.

Perform all steps in closed tubes. Fully fill tubes to prevent oxidation