

Standard aqueous clearing protocol for SmartSPIM following the [LifeCanvasWiki](#).

Solutions.

PBS with .02% Sodium Azide (PBS-SA)

SHIELD Epoxy Solution (SH-ES, freezer, 250 ml)

SHIELD Buffer Solution (SH-BE, RT, 125ml)

SHIELD ON Buffer (SH-ON, fridge, 500 ml)

Delipidation Buffer (DB-500, RT, 500 ml)

EasyIndex (EI-500-1.52, RT, 2 x 500ml)

Samples should be experience gentle rocking through this whole protocol. *Overall volumes needed for various sample sizes are as follows.*

	~Rat Brain	~Mouse Brain	~ 5 mm	~ 2 mm or smaller
Total volume	40 ml	20 ml	5 ml	2.5 ml

1) **Perfusion fixation.** For best tissue integrity, animals should transcardially perfused with cold heparinized PBS followed by cold 4% paraformaldehyde (PFA).

2) **Fixation.** Incubate samples in the fridge in 4% PFA in PBS for one day. Samples can then be stored in PBS-SA in the fridge until ready for use.

3) Incubate in SHIELD-OFF solution in fridge (4C).

SHIELD-OFF Solution recipe: 50% SHIELD-Epoxy, 25% SHIELD Buffer, 25% water

	~Rat Brain	~Mouse Brain	~ 5 mm	~ 2 mm or smaller
SHIELD OFF Incubation Time	6 days	3 days	2 days	1 day

4) **Incubate in SHIELD-ON solution** for 24 hours at 37C. If your sample is 2mm or smaller, see Wiki for slight change. Warm up SHIELD-ON solution before placing sample in it.

5) **Passive Delipidation.** Incubate in Delipidation Buffer at 45C. If you want to stain your sample with SYTO16 or Propidium Iodide, you can do this simply by adding it in a 1:1000 dilution (see other notes on nuclear staining in the Wiki). After this step, you can store for long periods of time in PBS-SA.

	~Rat Brain	~Mouse Brain	~ 5 mm	~ 2 mm or smaller
Delipidation Time	12 days	6 days	4 days	2 days

6) **Optional immunolabeling** (see wiki).

7) Index matching.

First incubate the tissue in 1:1 Easy Index:H2O at 37C.

Then incubate the tissue in 100% Easy Index at 37C.

	~Rat Brain	~Mouse Brain	~ 5 mm	~ 2 mm or smaller
Index Matching Time	2 days each	1 day each	12 hours each	6 hours each