ZerialLab

MAX PLANCK INSTITUTE
OF MOLECULAR CELL BIOLOGY
AND GENETICS

Fluorescence immunostaining – floating sections

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Reference: Morales-Navarrete et al., eLife (2019) 8:e44860; doi:10.7554/eLife.44860
Clearing modified after Ke et al. Nat Neurosci (2013) 16:1154–1161, doi: 10.1038/nn.3447

Buffer recipes

For better fixation and permeabilization, Tween can be added to PFA solution at 0.1% concentration at the end of preparation.

TxBuffer (1L):
0.2% gelatin (100mL 2% gelatin in PBS filtered, stored at -20˚C)
300mM NaCl (17.53g)
0.3% Triton X-100 (3mL)
Add PBS to make 1L, aliquot to 50mL falcon tubes, store at -20˚C

Heat up PBS, gelatin and NaCl to dissolve. After the solution has cooled below 40°C add Triton.

Modified SeeDB clearing

- M-SeeDB
80.2% (wt/wt) fructose, 0.5% 1-thioglycerol, ~0.1M phosphate buffer (pH7.5)*

- 100% Fructose pH7.5
100% (wt/v) fructose, 0.5% 1-thioglycerol, 0.1M phosphate buffer (pH7.5)

M-SeeDB recipe

Fructose	40.1 g
1M Na2HPO4	2.4 ml
1M NaH2PO4	0.6 ml
1-thioglycerol	0.15 ml
ddH2O	up to 50 g

100% Fructose

Fructose	40 g
1M Na2HPO4	3.2 ml
1M NaH2PO4	0.8 ml
1-thioglycerol	0.2 ml
ddH2O	up to 40 mL

To keep fluorescent signal and phalloidin staining, the solution has to be buffered.

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Method

Preparation of thick floating tissue section with Vibratome

Mold

- Turn on the water bath at 60 °C.
- Prepare 4 % agarose in PBS and maintain it melted in the bath water at 60 °C. Put agarose in the plastic embedding mold and add one piece of dry liver at the bottom.
- Make vibratome sections (50 to 200 µm thickness). Add PBS 500 µl/well in a 48 well plate. Just 1 slice per well.

Immunofluorescence

- Remove the agarose from the tissue and permeabilize with 0.5% Triton X-100 in PBS for 60 minutes (300 µl/well in 48 well plate) at RT.
- Add primary antibody in TxBuffer (2 overnights room temperature)
  One slice per well (24 well plate), 200 ~ 250 µl / well
  Flip the slice at day 1
  48 well plate, ~150 µl / well

3. Wash with 0.3% Triton/PBS 5 times for 15 minutes

4. Add secondary antibody + DAPI in TxBuffer (2 overnights room temperature)
   Upside down the slice at day1

5. Wash with 0.3% Triton/PBS (5 times 15 minutes)

6. Wash in PBS (3 times 1 minute)

7. M-SeeD Clearing.

- Add 200 ul of 25% fructose for 4 hrs,
then 50% fructose for 4 hrs ,
75% fructose ON
and 100% fructose ON.

Different concentrations of fructose are prepared diluting 100% fructose with water.

- Add 200 ul of SeeD ON. All these steps are at room temperature.

8. Mount on a glass slide with SeeD solution. #1.5 coverslips (thickness 0.17 ± 0.005 mm). RI:1,49

9. Immersion media: 80% 2,2'-Thiodiethanol. RI:1,49