IHC: Processing of mouse eye tissue for immunofluorescence microscopy

Solutions:

4% PFA-1XPBS (40mL)

10 mL 16% PFA

4mL 10x PBS

26 mL Water

-can keep for up to a month, store at 4°C

20% Sucrose (20mL)

5g Sucrose

2.5mL 10x PBS

Bring to 25mL with water

-can keep for up to a month, store at 4°C

o.1% Triton X-100 1xPBS

50ul PBS

450ul water

0.5mL

-Store at RT

Block Buffer 50mL

5 mL 10x PBS

5 mL Goat serum (10% final concentration)

2.5 mL 10% Trition x-100 (0.5% final concentration)

250 10% sodium Azide

\*use sterile water to bring volume to 50mL

-Store at 4°C

Antibody (Ab) Dilution

5 mL 10x PBS

2.5 mL Goat serum (10% final concentration)

2.5 mL 10% Trition x-100 (0.5% final concentration)

250 10% sodium Azide

\*use sterile water to bring volume to 50mL

-Store at 4°C

Preparing eye cups:

1. Remove eye by carefully cutting out or plucking deeply
2. Put eye directly into 4%PFA-1xPBS solution for 10 minutes
3. Poke hole in eye and remove cornea with microdissection scissors (Figure 1)
4. Agitate in 4%PFA-1xPBS for 50 minutes at RT
5. Wash with 1X PBS for 5 minutes x3

Cryoprotection:

1. Place eye in 20% sucrose-1XPBS in cold room, rocking, for a minimum for 3 hours but preferably overnight
2. Incubate in 1:1 solution of 20%sucrose-1x PBS and OCT for 30 mins
3. Removed lens (Figure 2) and incubate in 1:1 solution of 20%sucrose-1x PBS for an additional 30 mins
4. Place the eye cup in the cryomold facing up and fill mold with OCT
5. Gently Squeeze eye to remove air from the eye cup and position eye in the bottom right corner of the mold (Figure 3)
6. Look through the cryomold at eye level and make sure the eye is horizontal (Figure 4)
7. Freeze in the dry ice/100% ethanol bath by submerging the button of the cryomold into the ice bath (Figure 5)
8. Using a box of dry ice, transfer the eye cup to the -80°C for storage

Cutting:

1. Mount block on cryostat so that the small side of the rectangle is facing you (Figure 6)
2. Cut 30 micron sections until you can see the “C” shape of the eye up and then with a razor blade made a angled cut to removed excess OCT (reduces curling of cut sections) (Figure 7)
3. Collect 16 micron section, using cryostat, flatten sections with paint brushes
4. Place 3-4 section on a Superfrost+ slide
5. Let the slide dry at RT for 1 hour and store slides in -20°C

Staining: **Make sure sections do not dry out throughout this process**

1. Circle section with a pap-pan
2. Wash away OCT with 1X PBS for 5 minutes x3
3. Block for 1 hr. with blocking buffer at RT in a chamber
4. Suck up blocking buffer
5. Wipe off slide and reapply pap-pen
6. Combine primary antibody with Ab Dilution solution to yield the recommended concentration for the primary antibody
7. Apply primary antibody to slide at RT for 2hr-o/n, in a chamber
8. Wash for 15 minutes x2 in 0.1%Triton x-100 in 1XPBS in a jar
9. Wipe pap-pen off slide and re-apply pap-pen
10. Apply secondary antibody the same as primary, add 1ul of DAPI, apply on sections 1 hr, RT in a chamber
11. Suck out the Ab
12. Wash for 15 minutes x 2 with 0.1%Triton X-100 in1XPBS in a jar
13. Dry the slide and apply the mounting media, and place the cover slip. Seal perimeter with clear nail polish

Figure 1: enucleated eye with the cornea removed

Figure 2: enucleated eye with lens removed



Figure 3: Eye positioned in OCT within cryomold



Figure 4

Figure 5: eye cup being frozen in dry ice/ ethanol ice bath



Figure 6: OCT Block Mounted on Cryostat

Figure 7

Materials List:

* 16% PFA- Electron Microscopy Sciences (Cat. No. 15710)
* 10x PBS- Research products international (Cat. No. P32060-4000.0)
* Trition X-100- LabChem (Cat. No. LC262801
* Sucrose- Sigma (Cat. No. 84097-1kg)
* Goat Serum- Gibco (Cat. No. 16-210-064)
* Sodium Azide- GFS chemicals (Cat. No.3512)
* Pap pen- Research Products International
* Tissue Tek Cryomold 10mm X 10 mm X 5 mm (Cat. No.4565)
* Tissue Tek O.C.T. compound (Cat. No. 4583)
* Fluromount-G- Sountern Biotech (Cat. No.0100-01)
* Cover slips- Fisher 22x40-1.5 (Cat. No.12-544-B)
* Superfrost Plus Slides 25 X 75 X 1mm (Cat. No. 4951plus-001)
* Clear Nail polish