X-gal Staining of Wholemount *Drosophila* Embryos

Modified from The Little Blue Book (Gehring lab)

Uemura, 1995 July

Solutions

1. NaCl+TX:

   0.7% NaCl and 0.3% Triton

2. Fix Solution:

   3.7 % formaldehyde in 0.1 M phosphate buffer (pH 7.2)

3. PBT:

   0.1M Phosphate buffer (pH 7.2) and 0.3% Triton

4. Fe/NaP Buffer:

   3.1 mM $K_3Fe(CN)_6$
   3.1 mM $K_4Fe(CN)_6$
   10 mM NaPi (pH 7.2)
   0.15 M NaCl
   1.0 mM $MgCl_2$

   Shield a light.
   Add 0.3 % Triton before use.

5. X-gal Stock Solution:

   8% X-gal in Dimethyl formamide
   Aliquots should be stored at -20°C.

6. X-gal Working Solution:

   0.2% X-gal in Fe/NaP buffer

Staining of Embryos

1. Incubate Fe/NaP buffer (2ml/sample) an aliquot of X-gal in 37°C incubator for at least 30min.

2. With small brushes and NaCl+TX, collect embryos from grape plates and transfer to a watchglass. Wash embryos a couple of times by pipetting and aspirating NaCl+TX.

3. Dechorionate embryos with bleach for 3-4 min.

4. Rinse 3-4 times in NaCl+TX and once in dH_20.

5. Transfer embryos with a Pasteur pipet to a 20ml scintillation vial containing 5ml fix and 5ml n-heptane. Try to transfer as little liquid as possible to the fix. Fix for 20min at room temperature on a shaker platform at about 300rpm.

6. Remove heptane and fix solution from the vial (a 200 µl Gilson pipetman is suitable). To suck off the last 100-200 µl, press the pipet tip against the bottom of the tube so as to avoid removing embryos. Immediately wash at least twice with PBT, using the same technique to remove the solution. When the heptane has been washed away, the embryos no longer aggregate or stick to the sides of the tube.

7. Resuspend embryos in prewarmed X-gal working solution and incubate for 2 h to overnight at 37°C.

8. Rinse in NaCl+TX a couple of times so that embryos sink to the bottom of the vial. Rinse once in dH_20 and remove the liquid as much as possible.

9. Add 5ml n-Heptane first and then 5ml 100% methanol.

10. Shake the vial by hand for about 10 sec. The embryos will break out of their vitelline membranes and sink to the bottom of the vial.

11. Aspirate n-heptane. Rinse 1 times in methanol and 2 times in 100% ethanol.

12. Clear tissue with 2 rinses in xylene. Mount on a slide with 2-3 drops of ENTELLAN (MERCK). Allow to dry before viewing under high power.

X-gal Staining of Wholemount *Drosophila* Imaginal Discs

Uemura, 1995 July

Solutions

1. Fe/NaP Buffer:

   3.1 mM $K_3Fe(CN)_6$
   3.1 mM $K_4Fe(CN)_6$
   10 mM NaPi (pH 7.2)
   0.15 M NaCl
   1.0 mM $MgCl_2$

   Shield a light.

2. Fix Solution:

   0.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3)

3. X-gal Stock Solution:

   8% X-gal in Dimethyl Formamide
   Aliquots should be stored at -20°C.

4. X-gal Working Solution:

   0.2% X-gal in Fe/NaP buffer

Staining of Larval Tissues

1. Incubate Fe/NaP buffer and an aliquot of X-gal at 37˚C for at least 30 min.
2. Dissect 3rd instar larvae in PBS.
3. Fix tissues for 15 min at room temperature.
4. Wash in PBS containing 0.3% Triton for 10 min.
5. Wash twice in PBS and once in prewarmed Fe/NaP buffer.
6. Incubate in prewarmed X-gal working solution at 37˚C for 1 h to overnight.
7. Wash once in PBS.
8. Dissect imaginal discs and central nervous systems in PBS.
9. Mount samples on a slide with 2 drops of GEL/MOUNT.

Immunocytochemistry of Wholemount *Drosophila* Embryos (DAB)

Uemura, 1995 July

Solutions

1. NaCl+TX:

   0.7% NaCl and 0.3% Triton

2. Fix Solution:

   3.7 % Formaldehyde in 0.1 M phosphate buffer (pH 7.2)

3. PBT:

   0.1M Phosphate buffer (pH 7.2) and 0.3% Triton

4. DAB Stock Solution:

   50mg/ml Diaminobenzidine tetrahydrochloride (DAB) in dH_20
   Aliquots should be stored at -80°C.

5. DAB Working Solution:

   1mg/ml DAB in 0.12M Tris buffer (pH 7.5)

Staining of Embryos

1. With small brushes and NaCl+TX, collect embryos from grape plates and transfer to a watchglass. Wash embryos a couple of times by pipetting and aspirating NaCl+TX.

2. Dechorionate embryos with bleach for 3-4 min.

3. Rinse 3-4 times in NaCl+TX and once in dH_20.

4. Transfer embryos with a Pasteur pipet to a 20ml scintillation vial containing 5ml fix and 5ml n-heptane. Try to transfer as little liquid as possible to the fix. Fix for 20min at room temperature on a shaker platform at about 300rpm.

5. Pipette off the lower phase (fix). Add 5ml 100% Methanol and shake vigorously for about 10sec. The embryos will break out of their vitelline membranes and sink to the bottom of the vial. The membranes and any membraned embryos will remain at the interface.

6. Aspirate upper phase and most of the methanol. Rinse once in methanol and transfer the embryos with a pipette to a 24 well tissue culture plate.

7. Rinse 1-2 times in methanol, 3 times in 95% Ethanol and rehydrate with 70% EtOH and 35% EtOH. Then rinse 3 times in PBT.

8. Block for 1 hr in PBT containing 1-2% Bovine Serum Albumin (BSA). Add 0.5ml solution per well for subsequent steps.

9. Dilute primary antibody in PBT with 1-2% BSA. Incubate embryos overnight at

4˚C in the antibody solution.

10. Wash 4-5 times (10min each) in PBT.

11. Incubate 1hr at room temperature in (HRP-conjugated) secondary antibody diluted 1:100 in PBT with 1-2% BSA.

12. Wash 4-5 times in PBT, and then 3 times in 0.12M Tris buffer (pH 7.5).

13. Incubate 5-10min in 0.5ml DAB working solution. Add 0.5ml Hydrogen Peroxide diluted 1:1000 in dH_20. Watch the reaction under a dissecting microscope. When the signal is clear and before too much background appears, rinse 2-3 times in Tris buffer. Because DAB is a suspected carcinogen, it should be inactivated with bleach and disposed of properly.

14. Rinse 3 times in 100% ethanol. Clear tissue with 2 rinses in xylene. Mount on a slide with 2-3 drops of ENTELLAN (MERCK). Allow to dry before viewing under high power.

Immunocytochemistry of Wholemount *Drosophila* Embryos (fluorescence)

Uemura, 1995 July

Solutions

1. NaCl+TX:

   0.7% NaCl and 0.3% Triton

2. Fix Solution:

   3.7 % Formaldehyde in 0.1 M phosphate buffer (pH 7.2)

3. PBT:

   0.1M Phosphate buffer (pH 7.2) and 0.3% Triton

4. Mount stock solution:

   10mg/ml paraphenylenediamine in dH_20, PBS or TBS.
   Aliquots should be stored at -80°C.

5. Mount working solution:

   90% Glycerol and 1mg/ml paraphenylenediamine

Staining of Embryos

1. With small brushes and NaCl+TX, collect embryos from grape plates and transfer to a watchglass. Wash embryos a couple of times by pipetting and aspirating NaCl+TX.

2. Dechorionate embryos with bleach for 2-3min.

3. Rinse 3-4 times in NaCl+TX and once in dH_20.

4. Transfer embryos with a Pasteur pipet to a 20ml scintillation vial containing 5ml fix and 5ml n-heptane. Try to transfer as little liquid as possible to the fix. Fix for 20min at room temperature on a shaker platform at about 300rpm.

5. Pipette off the lower phase (fix). Add 5ml 100% Methanol and shake vigorously for about 10sec. The embryos will break out of their vitelline membranes and sink to the bottom of the vial. The membranes and any membraned embryos will remain at the interface.

6. Aspirate upper phase and most of the methanol. Rinse once in methanol and transfer the embryos with a pipette to a 24 well tissue culture plate.

7. Rinse 1-2 times in methanol, 3 times in 95% Ethanol and rehydrate with 70% EtOH and 35% EtOH. Then rinse 3 times in PBT.

8. Block for 1 hr in PBT containing 1-2% Bovine Serum Albumin (BSA).

9. Dilute primary antibody in PBT with 2% BSA. Incubate embryos overnight at 4˚C in the antibody solution.

10. Wash 4-5 times (10min each) in PBT.

11. Incubate 1hr at room temperature in (fluorescent dye-conjugated) secondary antibody diluted 1:100 in PBT with 2% BSA. Do not forget to wrap the tissue culture plate with aluminum foil.

12. Wash 4-5 times in PBT.

13. Mount embryos on slide glass. Place coverslips and seal with nail polisher.