

X-GAL STAINING [Adult Head Whole Mounts]

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DAY 1

- Anesthetize flies under CO₂ and remove heads with forceps.
- Transfer heads to an eppendorf tube containing 4% Paraformaldehyde/1X FIX BUFFER. Fix for 30 minutes on wet ice. Make sure that heads sink in fixative by agitating tube to encourage heads to sink.
- Remove fix. Wash 3 times 30 minutes, X-GAL BUFFER. If you are doing a lot of samples, fix and transfer to X-GAL BUFFER as you go along, then do 3 times 15 minute washes in X-GAL BUFFER.
- About ten minutes before the washes are done, prewarm X-GAL BUFFER to 37 degrees. Make up enough STAINING SOLUTION for 500 ul per tube. Add other components of staining solution and hold at 37 degrees until ready to incubate heads.
- Add 500 ul STAINING SOLUTION per tube. Incubate overnight at 37 degrees in a water bath or 37 degree incubator.

DAY 2

- Prewarm X-GAL BUFFER to 37 degrees.
- Remove staining solution and replace with 1 ml of prewarmed X-GAL BUFFER. Wash 3 times 15 minutes at 37 degrees (to discourage X-GAL crystal formation).
- Wash heads 5 minutes in 1XPBS.
- Remove most of 1XPBS and replace with 50% glycerol/1XPBS. Make 50% glycerol/1X PBS by mixing the following in a 50 ml tube:

25 ml glycerol

5 ml 10X PBS (Ca⁺², Mg⁺² free)

20 ml ultrapure water

- Mount heads in 50% glycerol/1X PBS on slides with bridge coverslips by transferring with wide bore pipet tips. Cover slips and photograph.

SOLUTIONS

1. FIXATIVE

Mix equal parts 2X FIX BUFFER and 8% PARAFORMALDEHYDE. Check final pH with a piece of pH paper to ensure that pH is approximately 7.0-8.0.

2. 2X FIX BUFFER

200 mM Sodium Phosphate pH7.4

10 mM EGTA

4 mM MgCl₂

100 mL

32.4 ml 0.5M dibasic Sodium Phosphate

7.6 ml 0.5M monobasic Sodium Phosphate

2 mL 0.5 M EGTA

0.4 mL 1 M MgCl₂

3. 8% PARAFORMALDEHYDE

Autoclave 450 mL ultrapure water in a 500 ml bottle. Transfer bottle to a fume hood, allow to cool for a few minutes, and add 40 g paraformaldehyde and 500 uL 10 N NaOH and a clean stir bar. Stir until dissolved on a magnetic stir plate, bring volume to 500 mL with ultrapure water and sterile filter. Good for 1 week if kept at 4 degrees.

4. X-GAL BUFFER

0.1 M Sodium Phosphate pH 7.4

2 mM MgCl₂

0.01% Sodium Deoxycholate

0.02% Nonidet P-40

1000 ml

162 ml 0.5M dibasic Sodium Phosphate

38 ml 0.5M monobasic Sodium Phosphate

2 ml 1 M MgCl₂

0.1 g

200 ul

5. STAINING SOLUTION

1 mg/ml X-GAL

1 mM Spermidine HCl

5 mM K₃Fe(CN)₆

Stocks

40 mg/ml

100 mM 50 uL

500 mM

For 5 mL

125 uL

500 uL

50 uL

For 50 mL

1.25 mL

500 uL

5 mM K₄Fe(CN)₆·6H₂O
X-GAL BUFFER

500 mM
1X

50 µL
to 5 mL

500 µL
to 50 mL