X-gal Staining on Adult Mouse Brain Sections

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[Abstract] Knowing expression patterns of given proteins is very important to understand their functions. Immunostaining analysis with specific antibodies is commonly used to identify cells or tissues expressing proteins of interest. Although this technique is regularly used, it requires high quality of specific antibodies and there is no good quality of antibody available for certain proteins. Alternatively, X-gal staining is also used to analyze protein expression pattern. It is simple and routinely used to detect expression pattern of any proteins of interest *in vivo*. In this method, genetically modified animals that express beta-galactosidase under the control of certain regulatory elements will be used to reveal the expression pattern of proteins that use the same regulatory elements.

Materials and Reagents

1. Adult mouse brain
2. Paraformaldehyde (PFA) (Sigma-Aldrich, catalog number: P6148)
3. Phosphate buffered saline (PBS)
4. Sucrose
5. Magnesium chloride, 6-Hydrate ($MgCl_2 \cdot 6H_2O$) (Mallinckrodt Baker, catalog number: 2444-01)
6. Sodium deoxycholate (Sigma-Aldrich, catalog number: D6750)
7. NP-40 (Sigma-Aldrich, catalog number: I3021)
8. Potassium Ferricyanide (ACROS ORGANICS, catalog number: 196785000)
9. Potassium Ferrocyanide (Mallinckrodt Baker, catalog number: 6932-04)
10. Permount (Fisher Scientific, catalog number: SP15-100)
11. O.C.T. compund (Sakura, catalog number: 4583)
12. Ethanol (Decon Labs, catalog number: 2716)
13. X-gal (American Bioanatycal, catalog number: AB00450-00005)
14. 1 M $MgCl_2$ (see Recipes)
15. 10% Sodium deoxycholate (see Recipes)
16. 20% NP-40 (see Recipes)
17. 50 mM Potassium Ferricyanide (see Recipes)
18. 50 mM Potassium Ferrocyanide (see Recipes)
19. Staining buffer (see Recipes)

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Equipment

1. Cryomold (Sakura, catalog number: 4557)
2. Cryostat
3. 37 °C incubator
4. 10-20 Slide Staining Dish with Cover (VWR International, catalog number: 900203) or equivalent

Procedure

1. Mouse is perfused with PBS for 5 min followed by 4% PFA/PBS for another 5 min.
2. Mouse brain is dissected out and post-fixed with 4% PFA/PBS for 4 h at 4 °C.
3. Fixed brain is washed with PBS three times and then incubated with 20% sucrose overnight (or until samples sink) and then 30% sucrose overnight.
4. The brain is mounted on standard cryomold with O.C.T. compound and stored at -80 °C until usage.
5. 40 µm cyro-section is made using cryostat and mounted on the slide-glass.
6. Slide-glass is washed once with the staining buffer (typically 150 ml for a container) for 10 min at room temperature.
7. Slide-glass is then incubated with 1 mg/ml X-gal in the staining buffer supplemented with 5 mM Potassium Ferricyanide and 5 mM Potassium Ferrocyanide at 37 °C until color develops.
   Notes:
   a. *This step usually takes 3 h to overnight.*
   b. *Don't let samples dry.*
   c. *Use 100-200 µl X-gal solutions per slide.*
8. Stained sample is washed with PBS three times (5 min each).
9. Slide is dehydrated with series of ethanol (50%, 75%, 90%, 100%, 2 min each).
10. Slide is mounted on permount and can be stored at room temperature.
11. Sample is ready for imaging at higher magnification (Figure 1).

Notes

1. The duration of incubation depends on how strongly beta-galactosidase is expressed. Sometimes it requires longer than 2 days of incubation. You can use fresh X-gal solution after overnight incubation.
2. Wet tissue must be put in the staining container.

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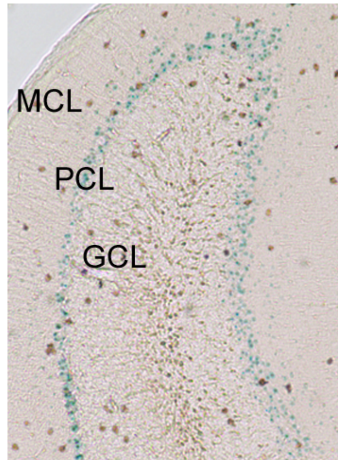

Figure 1. X-gal staining of *Nlk* gene trap mouse brain. Strong beta-galactosidase expression of the *Nlk* gene trap mouse is observed within the Purkinje cell layer from 6-week-old *Nlk* gene trap mouse cerebellum. MCL, molecular cell layer. PCL, Purkinje cell layer. GCL, granular cell layer.

Recipes

1. 1 M $MgCl_2$
   Add 10.17 g of $MgCl_2$ into 40 ml dH_2O
   Add dH_2O to 50 ml
   Stored at room temperature
2. 10% Sodium deoxycholate
   Add 5 g of Sodium deoxycholate into 40 ml dH_2O
   Add dH_2O to 50 ml
   Stored at room temperature
3. 20% NP-40
   Mix 10 ml NP-40 with 40 ml dH_2O
   Stored at room temperature
4. 50 mM Potassium Ferricyanide
   Add 16.46 g of Potassium Ferricyanide into 800 ml dH_2O
   Add dH_2O to 1,000 ml
   Stored at 4 °C
   Protect from light
5. 50 mM Potassium Ferrocyanide
   Add 21.12 g of Potassium Ferricyanide into 800 ml dH_2O
   Add dH_2O to 1,000 ml
   Stored at 4 °C
   Protect from light
6. Staining buffer

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Mix 2 ml of 1 M $MgCl_2$, 1 ml of 10% sodium deocycholate, 1 ml of 20% NP-40 with 800 ml dH_2O

Add dH_2O to 1,000 ml

Stored at room temperature

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