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Protocol status: Working
We use this protocol and it's working

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Gallyas Silver Staining

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ABSTRACT

Gallyas Silver staining in mouse brain sections stains degenerating neurites black.

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Day 1

1 For silver staining use the FD NeuroSilver^{TM} Kit II (FD NeuroTechnologies INC Cat# PK301)

2 Staining of free-floating brain sections is performed in glass staining dishes (Pyrex 36754-60) using 8-section staining nets (Ted Pella 36154-64)

2.1 The sections can be transferred between wells using a paint brush.

3 Wash sections 35µm mouse brain sections in 0.1M Phosphate buffer (PB) 3 times (5 minutes each wash) at room temp to remove cryoprotectant solution

4 Incubate sections in 0.1M PB + 4% paraformaldehyde pH 7.4 for 7 days at 4°C

4.1 Cover dish in parafilm

4.2 Lightly shake sections during this step - *we use a shaker in the cold room*

Day 8

5 *Perform all silver staining steps in a chemical fume hood.*

5.1 *Take shaker into fume hood for this purpose.*

5.2 *Collect all silver stain waste in large beaker for hazardous waste disposal*

6 Wash sections in miliQ H_2O 2 times for 5 minutes each wash at room temp

6.1 While sections are washing make up the A/B solution

6.2 Make 80ml per dish : 40ml sol A + 40ml sol B

7 Wash sections in A/B solution for 2 times for 10 min each wash at room temp

7.1 Use 25mL solution for each wash

8 Wash sections in A/B solution + E for 10 min at room temp

8.1 8mL sol A/B + 1 drop solution E

8.2 For 1 dish: 24ml sol A/B + 3 drops sol E

8.3 Wash sections in C/F solution for 2 min at room temp

8.4 50mL sol C + 2 drops sol F

8.5 Use 25mL per wash

9 Wash sections in C/F solution for 4 min at room temp

9.1 *Incubation longer than 4 min at this step decreases background and decreases signal. Shorter incubation increases signal but also increases background.*

10 Wash sections in D/F solution 1 time for 5 min at room temp

10.1 25mL sol D + 1 drop sol F

10.2 Make 25mL per dish

11 Wash sections in miliQ H_2O 2 times for 3 min each wash at room temp

12 Wash sections in 1X solution G 2 times for 5 min each wash

12.1 Dilute 10X sol G 1:10 in miliQ H_2O

12.2 Make 80mL per dish

12.3 Store sections in 1X sol G

13 Use a petri dish filled with 1X sol G to mount the sections on superfrost plus slides (Fisher Scientific 12-550-15)

14 This is easiest to do using a medium sized paint brush

15 Let sections dry at room temp after mounting

16 Protect slides from light

17 Clear sections in fresh xylene 3 times for 3 min each wash at room temp

17.1 **DO NOT** dehydrate in EtOH – it will cause loss of staining.

17.2 Use fresh xylene. Old xylene can contains residual EtOH from previous experiments. Residual EtOH will remove silver staining.

18 Use Entellan mounting medium (Electron Microscopy Sciences 14802) to coverslip slides

18.1 Apply one line of Entellan across the middle of the slide

18.2 Cover with glass coverslip and gently press down with forceps

18.3 Let slides dry in the fume hood for one hour – then dry on the bench overnight at room temp (protect slides from light) Gently remove excess Entellen with a razor blade