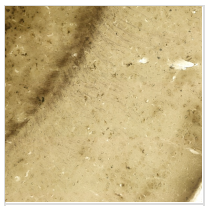

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Gallyas-silver stain

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Simplified Gallyas-silver staining protocol that works for pathological staining in rodent and human tissue

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Solutions that can be made in advance and stored in a fume hood

1 Make 4% of **NaOH** (sodium hydroxide) - use gloves, store in fume hood

1.1 1g ***NaOH*** + 25ml ***H20***

2 Make 2 x 600ml **dH20** – use gloves, store in fume hood

2.1 1.5g ***ammonium nitrate*** + 1.2g ***silver nitrate*** + 3.6g of 4%***NaOH***

Ammonium nitrate can cause an explosion with metals
Reaction between **silver nitrate** and ethanol is explosive
Reaction between **silver nitrate** and **NaOH** can form inflammable gases/vapors

3 Make **acetic acid** – use gloves, store in fume hood

3.1 0.5%: 3ml **acetic acid** in 597ml H20
0.1%: 0.6ml **acetic acid** in 600ml H20
0.05%: 50ml 0.5% **acetic acid** + 450ml H20

4 Make 0.2% **potassium ferricyanide** – can be stored up to a week, use gloves, store in fume hood

4.1 0.6g **potassium ferricyanide** in 300ml H20

5 Make 0.5% **sodium thiosulfate** - use gloves, store in fume hood

5.1 1.5g **sodium thiosulfate** in 300ml H20

6 Make 25%, 50%, 70%, 95% and 100% **ethanol**

Solutions that need to be prepared on the same day of staining

7 Make **pyridine + acetic acid** - use gloves, store in fume hood

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7.1 100ml **pyridine** + 50ml **acetic acid**

Pyridine is incompatible with rubber, plastics, and metals! Very important to use butyl-rubber gloves with this chemical

8 Make **silver nitrate** (add in this order) - use gloves, store in fume hood

8.1 100ml **dH20**
0.192g **ammonium nitrate**
0.2g **silver nitrate**
0.6ml of 4% **NaOH**
pH must be 7.5

Ammonium nitrate can cause an explosion with metals
Reaction between **silver nitrate** and **ethanol** is explosive
Reaction between **silver nitrate** and **NaOH** can form inflammable gases/vapors

9 Make **A + B + C** (1L) - use gloves, store in fume hood

9.1 **Solution A**
5g anhydrous sodium carbonate + 100ml dH20

Solution B (add in this order)
100ml dH20 + 0.19g ammonium nitrate + 0.2g silver nitrate + 1g silicongulistic (tungolistic) acid

Solution C (add in this order)
100mldH20 + 0.19g ammonium nitrate + 0.2g silver nitrate + 1g silicongulistic (tungolistic) acid + 0.66ml 37% formaldehyde

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A + B + C - use gloves, store in fume hood
50ml **A** + 37.5ml **B** + 37.5ml **C** (25±2°C)

Ammonium nitrate can cause an explosion with metals
Reaction between **silver nitrate** and **ethanol** is explosive
Reaction between **silver nitrate** and **NaOH** can form inflammable gases/vapors
Formaldehyde may cause cancer

Consumables for staining

10 17 x glass petri dishes
20 x glass pipettes
1 x rubber pump for glass pipettes
Butyl rubber gloves
Lab coat with long arms
Protective eyewear
Face mask

Need to use glass petri dishes and glass pipettes for each wash

Gallyas-silver staining

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Step	Reagent	Minutes	Comments
1	***dH20***	3 or more	Put in dH20 while preparing other solutions
2	***Pyridine + acetic acid***	60	On shaker
3	***50% ethanol***	3	
4	***25% ethanol***	3	
5	***0.05% acetic acid***	2.5	
6	***0.1% acetic acid***	2.5	
7	***0.05% acetic acid***	10	Leave longer if necessary, to prepare silver nitrate
8	***Silver nitrate***	60	On shaker – make A+ B + C
9	***0.5% acetic acid***	10	

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10	***A + B + C***	6 (15min human)	Do not let the tissue become dark, time is dependent on sample
11	***0.5% acetic acid***	1	New
12	***0.2% potassium ferricyanide***	5	On shaker
13	***dH20***	1	
14	***0.5% acetic acid***	1	New, can be reused
15	***0.5% sodium thiosulfate***	2	On shaker
16	***dH20***	4	
17	***dH20***	4	
18	***0.5% acetic acid***	1	Use the one from the previous step
19	***A + B + C***	3-4	On shaker
20	***0.5% acetic acid***		Use the one from the previous step
21	***0.2% potassium ferricyanide***	3	On shaker
22	***dH20***	1	
23	***0.5% sodium thiosulfate***	2	On shaker
24	***dH20***	4	
25	***dH20***	4	
26	***0.5% acetic acid***	1	New, can be reused
27	***A + B + C***	3-4	On shaker
28	***0.5% acetic acid***	1	Use the one from the previous step
29	***0.2% potassium ferricyanide***	10	On shaker
30	***dH20***	1	
31	***0.5% sodium thiosulfate***	2	On shaker
32	***dH20***	Fast wash	
33	***dH20***	5	
34	Dehydrate	3 in each	50% - 70% -95% - 100% - 100% ethanol
35	***Xylene x 2***	3 in each	
36	Coverslip		

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