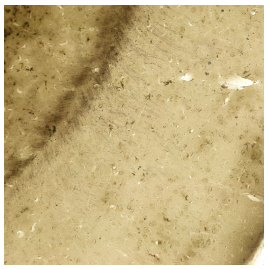
Feb 15, 2022

Gallyas-silver stain



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

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Keywords: pathological staining in rodent, silver staining protocol, pathological staining, silver stain, gallya, staining protocol, rodent, human tissue

Abstract

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

Troubleshooting

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 × 600ml **dH20** – use gloves, store in fume hood

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

Safety information

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

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

Safety information

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

Safety information

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

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

Safety information

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

Note

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	
	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		