

Title: Fontana-Masson staining for neuromelanin

Description of activities:

1. Make up 1 mL of 10% silver nitrate in distilled water.
2. Add concentrated ammonium hydroxide to the solution dropwise until the precipitate almost dissolves - should be cloudy and a faint opalescence should be observed.
3. Add a further 1 mL of distilled water and filter the working solution. The solution will last for about 4 weeks if stored in a dark bottle.
4. Air dry sections onto clean optical microscopy slides, and encircle sections using a PAP pen.
5. Hydrate the tissue sections by dropping 3 uL distilled water over the sections. Wick off excess using filter paper.
6. Treat sections with silver nitrate working solution (3 uL per section) and leave overnight in the dark.
6. Wick off silver solution using filter paper and wash well with several changes of distilled water.
7. Treat sections with 5% sodium thiosulphate for 2 minutes.
8. Counterstain with 1% neutral red (aqueous) for 3 minutes.
9. Wash with distilled water.
10. Dehydrate sections using graded alcohols up to xylene.
11. Mount using DPX medium and coverslip the slide for optical microscopy.

Location: F012B

Persons at risk: Anyone carrying out the staining protocol

Hazard 1: Silver nitrate is an oxidiser and may cause fire if exposed to an ignition source. Ethanol, xylene and DPX mounting medium is also highly flammable.

Existing control measures: Keep all materials away from ignition sources by carrying out work in fume hood.

Additional control measures: Minimise quantities used. Ensure sand is present to extinguish fire if necessary.

Severity of injury: serious

Likelihood: Unlikely

Risk factor: Low

Hazard 2: Silver nitrate and ammonia may cause skin burns and eye damage.

Existing control measures: Work carried out in fume hood to minimised exposure.

Additional control measures: Wear gloves and eye protection to avoid contact.

Severity of injury: Major

Likelihood: Unlikely

Risk factor: Low

Hazard 3: Exposure to ammonia or xylene may cause respiratory irritation

Existing control measures: Work carried out inside fume hood to avoid inhalation.

Severity of injury: Major

Likelihood: Unlikely

Risk factor: Low

RECORD OF PERSONNEL INVOLVED

Name	Position and Qualifications	Experience	Start Date	Finish Date
Jake Brooks	PDRA	6 year's lab experience	Oct 2016	-

Reviewed 11/05/2021: newer members of the laboratory will need to become familiar with the content so that they can be formally added to this approval by Prof. Collingwood before performing the procedure.



11th May 2021