

INTERNAL SAFETY RULES : APPENDIX

Sample staining using uranium solutions for electron imaging

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1 Disclaimer

The following procedures should be used as a template only and is based on the preparation of the staining agents and their use in electron imaging. The specific details may have to be slightly modified to address the particular laboratory environment and needs but should always follow the specific rules and practices established in the safety rules document "Sample staining using uranium solutions for electron imaging" [1]. All of the information on this document is provided "as is", without warranty of any kind, either express or implied.

2 Examples

2.1 Preparation of the uranyl acetate staining solution

The procedure provides the final user with 1 ml of uranyl acetate staining solution at 1%. This activity must be carried in a C-laboratory accredited for the use of uranium. In addition to the protection equipment (see [1], section 3.3), the following equipment is used:

PPE

- lab coat,
- gloves,
- safety glasses.

Equipment

- a fume hood,
- a precision weighing balance,
- an ultrasonic bath.

Material

- absorbent laboratory surface protection paper,
- radioactive warning labels,
- a disposable plastic spatula,
- a narrow-mouth amber glass bottle (10 ml) or a glass scintillation vial,
- a 7 mL square weighing boat,
- paper or tissue (for cleaning),
- a plastic container for dry, uranium-contaminated waste.

Chemicals

- 50 mg uranyl acetate dihydrate powder,
- 5 ml double-distilled water (ddH₂O).

Steps

1. Wear appropriate PPE (lab coat, safety glasses, gloves).
2. Cover work area (within the dedicated fume hood) with the lab surface protection paper and label it appropriately (see figure 10 in reference document [1]).
3. Place a weighing boat on the balance and tare the balance inside the dedicated fume hood.
4. Weigh out 50 mg of uranyl acetate powder using a plastic spatula.
5. Put the powder into a glass scintillation vial.
6. Measure 5 mL of double distilled water in a cylinder and add to the uranyl acetate powder in the scintillation vial.
7. Throw the spatula and the weighing boat into the contaminated waste.
8. Close the scintillation vial and place it in an ultrasonic bath until it is completely dissolved.
9. Gather and dispose of all contaminated material as radioactive waste in the appropriate container.
10. Clean workplace area (incl. wipe down surfaces and material). Replace surface protective paper at least once per month (note down date) or earlier in case of a spill.
11. Dispose of all cleaning material as radioactive waste.
12. Discard gloves and wash hands thoroughly.

2.2 Preparation of the uranyl formate staining solution

The procedure provides the final user with 1 ml of uranyl formate staining solution at 1%. The solution can be used for 2-3 days. This activity must be carried in a C-laboratory accredited for the use of uranium. In addition to the protection equipment (see [1], section 3.3), the following equipment is used:

PPE

- lab coat,
- gloves,
- safety glasses.

Equipment

- a fume hood,
- a hot plate,
- a magnetic stirrer and a stirring bar (but not the same as the already mentioned hotplate),
- a precision weighing balance,
- 10 µl and 1000 µl transfer pipettes.

Material

- absorbent laboratory surface protection paper,
- radioactive warning labels,
- Parafilm[®],
- a disposable plastic spatula,
- a 200 ml beaker covered with aluminum foil,
- a 50 ml glass beaker,
- a 2 mL Eppendorf tube,
- a 10 ml narrow-mouth bottle amber glass,
- a syringe, a syringe filter 0.2 µm and a needle,
- paper or tissue (for cleaning),
- a plastic container for dry, uranium-contaminated waste.

Chemicals

- 20 mg of uranyl formate dihydrate powder,

- double-distilled water (ddH₂O),
- 3 μl 5 M sodium hydroxide (NaOH) solution.

Steps

1. Wear appropriate PPE (lab coat, safety glasses, gloves).
2. Cover work area (within the dedicated fume hood) with the lab surface protection paper and label it appropriately (see figure 10 in reference document [1]).
3. Boil more than 2 mL of ddH₂O using a hot plate or microwave.
4. Measure out 20 mg of uranyl formate in a 50 mL glass beaker under a dedicated fume hood.
5. Add 2 mL of hot water to the beaker containing the uranyl formate powder in the dedicated area within the fume hood and stir it for 5 to 10 minutes until it is dissolved. Keep the solution protected from light using a 200 mL beaker covered by an aluminum foil.
6. While continuing to stir, add 3 μL of 5 M NaOH and keep it stirring for 5 to 10 more minutes. The solution should be yellow in color.
7. Use a syringe with a clean needle to collect all the solution.
8. Remove and discard needle safely in a dedicated sharps' container.
9. Add the 0.2 μm syringe filter to the tip of the syringe.
10. Filter and collect the solution in the final bottle.
11. Store the uranyl formate staining solution protected from light. Discard the stain into a radioactive liquid waste container if any precipitate or a mild discoloration is observed.
12. Clean workplace area (incl. wipe down surfaces and material). Replace lab surface protective paper at least once per month (note down date) or earlier in case of a spill.
13. Dispose of all cleaning material as radioactive waste.
14. Discard gloves and wash hands thoroughly.

2.3 Examples of procedures for samples staining

Two different staining procedures are commonly used at EPFL. They mostly differ at the step of cleaning the sample of the staining solution excess.

2.3.1 Wet cleaning method

In addition to the protection equipment (see [1], section 3.3), the following equipment is used:

PPE

- lab coat,
- gloves,
- safety glasses.

Equipment

- a rotator.

Material

- absorbent laboratory surface protection paper,
- MatTek dish™,
- a silicon plate for holding grids,
- a water squeeze bottle,
- a glass pipette,
- paper or tissue (for cleaning),
- a plastic container for dry, uranium-contaminated waste.
- a plastic container for liquid uranium waste,

Chemicals

- uranyl acetate 1% staining solution,
- double distilled water.

Procedure for inclusion of samples, cells and tissues

1. Wear appropriate PPE (lab coat, safety glasses, gloves).
2. Cover work area (within the dedicated fume hood) with the lab surface protection paper and label it appropriately (see figure 10 in reference document [1]).
3. Rinse the samples in double distilled water. This process can be done in a scintillation vial for free floating samples or in a MatTek™ dish in the case of adherent cells.
4. Remove all double distilled water and directly add the uranyl acetate 1% staining solution within the dedicated area.
5. Continuously mix the samples for 30 to 40 minutes at room temperature by placing the scintillation vial or culture dish on a rotator.
6. After staining in uranyl acetate, remove the solution completely and wash the sample with double distilled water several times (at least three time), first and second wash are discarded in the liquid radioactive uranium waste container. Subsequent washes are discarded in aqueous waste (non-radioactive).
7. Clean workplace area (incl. wipe down surfaces). Replace lab surface protective paper at least once per month (note down date) or earlier in case of a spill.
8. Dispose of all cleaning material as radioactive waste.
9. Discard gloves and wash hands thoroughly.

Procedure for grids staining

1. Wear appropriate PPE (lab coat, safety glasses, gloves).
2. Cover work area (within the dedicated fume hood) with the lab surface protection paper and label it appropriately (see figure 10 in reference document [1]).
3. Place the grids into the vertical slots of the silicon staining plate.
4. Flood the plate, using a water squeeze bottle with double distilled water.
5. Remove the water with the glass pipette and then directly add the uranyl acetate 1% solution on the grids at room temperature.
6. After 10 minutes, remove the liquid completely and wash at least three time with double distilled water, first and second wash are discarded in the liquid radioactive uranium waste container. The subsequent washes are discarded in aqueous waste (non-radioactive).
7. Dry the grid with a filter paper.
8. Clean workplace area (incl. wipe down surfaces). Replace lab surface protective paper at least once per month (note down date) or earlier in case of a spill.
9. Dispose of all cleaning material as radioactive waste.
10. Discard gloves and wash hands thoroughly.

2.3.2 Dry cleaning method

In addition to the protection equipment (see [1], section 3.3), the following equipment is used:

PPE

- lab coat,
- gloves,
- safety glasses.

Equipment

- No specific equipment required.

Material

- absorbent laboratory surface protection paper,
- Parafilm®,
- a pair of tweezers,
- grids for TEM,
- a micropipette and its tips,
- filter paper,
- a plastic container for dry uranium-contaminated waste,
- a plastic container for liquid uranium waste,

Chemicals

- uranyl acetate 1% staining solution.

Steps

1. Wear appropriate PPE (lab coat, safety glasses, gloves).
2. Cover work area (within the dedicated fume hood) with the lab surface protection paper and label it appropriately (see figure 10 in reference document [1]).
3. Cut a piece of Parafilm® and place it on the workbench.
4. Prepare cuts of filter paper and display them close to the parafilm.
5. Place a drop of 12-14 µl of sample solution on the parafilm.
6. Place the grid on the drop for 1-2 minutes.
7. Wash the grid with several drops of buffer solution (or water) deposited on the parafilm.
8. Place a drop of 12-14 µl of staining solution on the parafilm.
9. Place the grid containing the adsorbed sample on the drop of uranyl acetate for 20-30 seconds.
10. Dry the grid off with filter paper.
11. Dry the grid at room temperature while mounted on the tweezer.
12. Repeat steps 3 to 9 for another grid if necessary.
13. Dispose of the remaining drop of staining solution in the liquid radioactive uranium waste container.
14. Dispose of the filter paper, the parafilm and the tip of the micropipette and any other contaminated materials in the solid radioactive uranium waste container.
15. Clean workplace area (incl. wipe down surfaces). Replace lab surface protective paper at least once per month (note down date) or earlier in case of a spill.
16. Dispose of all cleaning material as radioactive waste.
17. Discard gloves and wash hands thoroughly.

3 List of products

3.1 Protective surface absorbent paper

- Separate sheets, Fisher Scientific Cytiva Whatman™ Benchkote Surface Protector Sheets, reference [11300674](#) (113.- CHF for 50 sheets);
- Roll, Fisher Scientific Fisherbrand™ Grade 604 Surface Protection Paper, reference [11758722](#) (188.- CHF for 50 m);

4 References

- [1] EPFL DSPS-SCC, "Sample staining using uranium solutions for electron imaging," *Internal Safety Rules*, 2021.