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A Stable Lead Citrate Stain for Grids

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For people in a small EM lab, keeping stable stain solutions that don't accumulate precipitates over time can be a problem. Over the last 26 years, I have adapted the method originally described by Venable and Coggeshall *(1)* to address this need.

Materials:

Lead Citrate powder

10N NaOH

Deionized or double distilled water

20 ml syringe with a cap for the tip

PVDF membrane syringe filter with 0.2 micrometer pores

~0.01N NaOH solution (carbonate-free) for rinsing grids (I make this up by adding 2 pellets of NaOH to 500ml deionized water)

Method:

Heat 15ml water on a hot plate until bubbles are released, but do not allow it to reach a full boil. Let it stand to cool slightly and retain 10 ml.

Weigh out 0.02 g lead citrate powder.

Add the lead citrate powder to the warm, degassed water and swirl gently to mix, taking care not to agitate the water thus introducing air.

Add one drop (from a 1.5 ml transfer pipet) of 10N NaOH* and continue to swirl. This will adjust the pH and aid mixing. Do not worry if all of the lead citrate doesn't dissolve. The original paper *(1)* used a range of 0.01-0.04 gm/10 ml.

Draw the solution up into the syringe and expel any air. Cap the syringe, wrap it in foil and store at room temperature.

How to Use:

Prepare a staining chamber by placing a square of Parafilm (American National Can Co.) in the bottom portion of a 100 mm diameter plastic tissue culture dish. Place approximately 20 pellets of NaOH in the dish, beside the Parafilm. This will absorb water and carbonates from the air inside the chamber, helping to prevent precipitates.

Remove the syringe from the foil and replace the cap with a filter. Dispense the first drop as waste, then lay out an array of drops on the Parafilm. I usually avoid staining more than 4 sets of grids at a time, to minimize the chances of contamination.

Lay grids, sections down, on the drops of stain. Take care not to breathe on the stain. *(I lift the lid as if I was feeding cultured cells, keeping the top of the lid tilted toward me).* If you are not staining serial sections, 3 or 4 grids of the same sample can be placed on a single drop. Replace the cover on the chamber.

Stain for 5-6 minutes.

Using the same lid-lifting technique, pick a single grid from a drop of stain and immediately dip repeatedly (about 20 times) into a beaker of the 0.01 N NaOH. This will wash off the stain while minimizing precipitates. Wash the grid with deionized water, either by dipping or with a gentle stream from a wash bottle and use filter paper to blot between the tips of the forceps and the edge of the grid. Place on a clean piece of filter paper, or hold in the forceps until dry.

This stain may be used after staining with uranyl acetate (UA), or alone if the tissues have received *en bloc* staining with UA. I have used this technique with much success for well over 20 years.

The formulation of the stain is that described by Venable and Coggeshall in their original article *(1)*. My adaptations are the storage conditions and the initial wash in the dilute NaOH. ■

References

Venable, J.H. and Coggeshall, R. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* 25, 407-408, 1965.

(*As with Reynold's Lead citrate, excess NaOH will raise the pH too much and result in a negative contrast effect).

Microscopy AND Microanalysis

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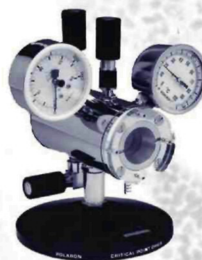

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