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Reynolds' Lead Citrate Stain

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ABSTRACT

Reynolds Lead Citrate Stain for electron microscopy is a standard stain developed by Edward S. Reynolds and consists of a mixture of 80 mM Lead(II) nitrate and 120 mM Sodium citrate. Sodium hydroxide is added to make the stain basic to pH 12.

Preparation time:

40-60 min

Reference:

Edward S. Reynolds; THE USE OF LEAD CITRATE AT HIGH pH AS AN ELECTRON-OPAQUE STAIN IN ELECTRON MICROSCOPY .*J Cell Biol* April 1963; 17 (1): 208–212. doi:<https://doi.org/10.1083/jcb.17.1.208>

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Protocol status: In development
We are still developing and optimizing this protocol

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GUIDELINES





This protocols aim to show the exact method that the Centre for Cellular Imaging at the University of Gothenburg use to prepare a standard buffer.

We do not claim to have discovered, invented or otherwise hold any form of intellectual property of the buffer(s) described in this protocol.

Feel free to visit us: <https://www.gu.se/en/core-facilities/centre-for-cellular-imaging>

MATERIALS

Chemicals

-  Lead(II) nitrate (EMSURE®) **Sigma Aldrich Catalog #1073980100**
-  Tri-Sodium citrate dihydrate (EMSURE®) **Sigma Aldrich Catalog #1064480500**
-  Sodium hydroxide BioXtra $\geq 98\%$ (acidimetric) pellets (anhydrous) **Sigma Aldrich Catalog #S8045-1KG**
-  Milli-Q water **Contributed by users**

Consumables

- 50 ml volumetric flask
- Magnetic stirrer bead
- 5 x 10 ml syringes
- 10 ml pipette
- Pasteur pipette
- Parafilm
- Weighing paper
- Weighing spatula

Equipments/Machines

- Scale
- Magnetic stirrer

SAFETY WARNINGS

- ! Use caution, work in a fume hood, wear gloves, lab coat, and goggles.

⊗ Lead(II) nitrate (EMSURE®) **Sigma Aldrich Catalog #1073980100**

H272 May intensify fire; oxidizer.

H302 + H332 Harmful if swallowed or if inhaled.

H317 May cause an allergic skin reaction.

H318 Causes serious eye damage.

H360FD May damage fertility. May damage the unborn child.

H372 Causes damage to organs (Blood, Central nervous system, Immune system, Kidney) through prolonged or repeated exposure.

H410 Very toxic to aquatic life with long lasting effects.

⊗ Tri-Sodium citrate dihydrate (EMSURE®) **Sigma Aldrich Catalog #1064480500**

Not a hazardous substance or mixture according to Regulation (EC) No 1272/2008.

⊗ Sodium hydroxide BioXtra ≥98% (acidimetric) pellets (anhydrous) **Sigma Aldrich Catalog #S8045-1KG**

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

BEFORE START INSTRUCTIONS






Book a fume hood and label the syringes for aliquots.

50 ml Lead citrate for Array tomography




31m


1 Weigh out and dissolve Lead (II) nitrate and tri-Sodium citrate dihydrate:

31m

- Weigh out  1.32 g of **⊗ Lead(II) nitrate (EMSURE®) Sigma Aldrich Catalog #1073980100** in a 50 ml volumetric flask in which you have added a small magnetic stirrer.
- Dissolve the Lead(II) nitrate by adding  30 mL of **⊗ Milli-Q water Contributed by users** and turning on the stirrer.
- Weigh out  1.76 g of **⊗ Tri-Sodium citrate dihydrate (EMSURE®) Sigma Aldrich Catalog #1064480500** in a small weighing boat. Add it to the dissolved Lead(II) nitrate.
- Shake the mixture vigorously, or vortex it for  00:01:00 The solution will be milky.
- Cover the volumetric flask with parafilm and let it stir for an additional  00:30:00

Prepare a 1 M sodium hydroxide (NaOH) solution:

- Add  10 mL of  Milli-Q water **Contributed by users** to a 15 ml falcon tube.
- Weigh out  0.4 g of

 Sodium hydroxide BioXtra $\geq 98\%$ (acidimetric) pellets (anhydrous) **Sigma Aldrich Catalog**
#S8045-1KG



on a small weighing paper, and add the NaOH to the water.

- Mix the NaOH until it dissolves by inverting the falcon tube.


Note

If your lab have a stock solution of NaOH, this can be diluted to 1 M and used instead of preparing it fresh as described above.


Finalizing the Lead citrate solution:

- Add  8 mL of the 1 M NaOH solution to the Lead(II) nitrate/Tri-Sodium citrate mixture, this will raise the final pH to 12 ± 0.1 .
- Keep stirring until the solution is clear.
- Add  Milli-Q water **Contributed by users** until the volume is 50 ml.

Aliquot the mixture in:

5 x  10 mL - 10 ml syringes labelled: *Lead citrate and date*.

Storage:

Mix on the day of use for optimal staining. For longer storage, seal syringes with parafilm and store long term (4 weeks) at  4 °C

Note

If using stored lead citrate, carefully inspect before usage for precipitates. Do not use if precipitates are formed. Always add a 0.2 μm filter to the syringe before use.

Clean up:

Rinse the 50 ml volumetric flask and magnetic stirrer twice with water, pouring the wash of in a liquid lead waste. Then rinse thoroughly with water before putting them in the dishwasher.