PROCEDURE:

Preparing Osmium Tetroxide (OsO₄) from Crystalline Form

Are you looking for an easy SOP for preparing osmium tetroxide (OsO₄) from crystalline form? Then look no further! Osmium tetroxide is a rare chemical used as a fixative and a staining agent for biological samples examined under electron microscopes. Osmium tetroxide is typically colorless or pale yellow, though it can also be a white crystalline solid.

Remember to follow all safety requirements when handling OsO₄.

- 1. Determine the final concentration, vehicle and volume of Os solution desired. NOTE: When making Os from a crystal, the entire crystal must be used at the same time.
- 2. Determine the mass of OsO₄ crystal in the sealed ampoule. They are typically offered in weights of 1, 2, or 4 grams.
- 3. Using an appropriate size storage container, with a tight sealing top, rinse thoroughly to ensure cleanliness.
- **4.** Measure and add volume of vehicle to the clean storage container.
- 5. Wearing gloves, eye protection, and in the fume hood, remove ampoule from container and wipe clean using a lint free cloth.

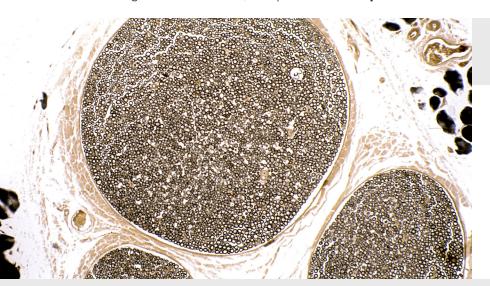
NOTE: Paper particles react and reduce OsO₄, making it ineffective.

- 6. Using an ampoule breaker, snap off the pre-scored top and drop both pieces into the storage container. Use a pipette to ensure that the liquid fills the ampoule.
- 7. Secure the lid, wrap well with parafilm, and store in air tight box in the fume hood for four (4) hours.

NOTE: Step 7 takes at least 24 hours to dissolve into solution.

NOTE: To decrease dissolving time, try one of the following methods:

- **A.** Place the container in a sonicator, in the fume hood.
- **B.** Rotate the ampoule under hot water to melt the crystal. When the crystal melts, remove from the hot water and continue turning the ampoule while a thin coating forms on the inside, then proceed from **step 6** above.







EMS Catalog supplies used

0s04, Crystalline

1g	Cat. No. 19110 (10 x 1g)
2g	Cat. No. 19112 (5 x 2g)
4g	Cat. No. 19114 (5 x 4g)

Ampoule Breakers

Cat. No. 60600, 60605-04, 60607

Parafilms

Cat. No. 70990, 70991, 70994-01 series

Examples of Common Dilutions

1 gram into 25 ml = 4% 2 grams into 50 ml = 4% 1 gram into 50 ml = 2% 2 grams into 100 ml = 2%

Cross section of a nerve fascicle fixed in osmium tetroxide. Each small black circle inside the fascicle corresponds to a myelinated nerve fiber.

Image credit: Jose Luis Calvo.











