PELCO®

Uranyl Acetate- Lead Citrate Instructions
SynapTek™ GridStick™ Staining

Product No. 155

You will need the following materials:

SynapTek™ GridStick™ Kit

GridSticks (5): Each kit contains five GridSticks stored inside five pipettes with red caps.

Pasteur Pipettes (10): Each kit contains ten pipettes, five for Uranyl Acetate and five for Lead Citrate. These pipettes have a short tapered neck so the caps will fit, and a large diameter tip to minimize pressure changes during staining.

Rubber Bulbs, and Plugs: We include two rubber bulbs with flow-limiting plugs. These plugs also keep debris and dirt from entering the pipette, and can be used to hold NaOH pellets during lead staining.

10% Uranyl Acetate

10 gm Uranyl Acetate 100 ml methanol: Place solution in a glass flask with a glass cap, and sonicate or shake until totally dissolved. You may want to filter or spin down, but we find it is usually not necessary. Store in dark freezer or refrigerator, but use solution at room temperature.

100% Methanol

Three methanol washes of 5-10 ml each.

0.1 % Lead Citrate

0.1 gm lead citrate
100 ml freshly distilled water.
Add 1 ml drop by drop of 10 N NaOH (0.4gm NaOH per 1ml) after lead has dissolved in water until the solution is clear. Store in airtight containers such as our 12ml Sample Vial (Product No. 413) at room temperature.

NaOH Pellets

NaOH pellets may be inserted into the rubber bulb to insure a carbon dioxide free pipette during lead staining.

0.1 N NaOH Wash

Make a l0ml 0.1 N NaOH wash solution and adjust PH to 12 using HCL.

100 cc of freshly distilled water

A clean glass beaker

PROCEDURE:

Step1--Place up to ten grids on the stick. If you are using formvar-coated grids, be sure that the formvar is away from the GridStick's surface and that the slot runs in the same direction as the GridStick's slot. It helps to place the stick on a clean, flat surface and to push down with forceps on the outer edge of the grid against the adhesive to insure good contact. If you are using thin mesh grids, it is only necessary to lightly push on the outer rim. Although the pipettes have been treated to remove all organic residues as well as washed and blown dry with filtered air, it is always a good practice to rinse the pipette with a few methanol washes before starting.

Step 2--Place the GridStick™ inside the first pipette and insert the rubber bulb with red plug in the pipette's top. Push the bulb's rubber rim down around the pipette to prevent accidental release. The red plug has a very small hole on its side and serves two important functions: First, it prevents any dust panicles from entering the pipette; and second, it limits the rate of flow of staining and washing solutions, thereby limiting pressure changes so that formvar films do not break. Gently aspirate one to two cc's of the uranyl acetate solution into the pipette so that all grids are fully immersed, placing the tip in an empty beaker. Do not leave pipette in uranyl acetate beaker or the solution will be aspirated into the rubber bulb. Place in dark or subdued light for 10-30 minutes.

Step 3--Dispose of uranyl solution and rinse at least three times in separate methanol washes.

Step 4--Remove GridStick™ from Pipette One and place in a petri dish or some other dust-free area to dry about 30 minutes. You can leave it in the pipette, but drying time will increase to about one hour.

Step 5--After grids are totally dry (very important that they **are dry)** place the SynapTek^{TM} GridStick^{TM} in Pipette Two and gently aspirate one to two cc's of the NaOH wash solution so that all grids have been immersed at least once. Since water has a high surface tension it is important that all aqueous solutions be aspirated slowly. If you become too careless in this step you may find small holes in your formvar films.

Step 6--The NaOH wash will purge the pipette of CO_2 and is usually more than adequate; but if you like, you may also place a few pellets of NaOH in the second rubber bulb, and pump it several times. No CO_2 will remain in the pipette. Gently aspirate the lead staining solution in the just-washed pipette and stain for three minutes.

Step 7--Wash once again in the 0.1 N NaOH.

Step 8--Wash the grids in a 100 cc beaker of distilled water at least a dozen times. Be careful and go slowly. One effective wash method is to remove the rubber bulb and simply dip the entire pipette with grid stick inside, into the beaker. Lift the pipette out of the beaker and let the water drain, and so on for a dozen or more times.

Step 9--Remove SynapTek^{TM} GridStick^{TM} from pipette and let grids dry

Step 10-- Remove GridStick™ and place in a pipette with red caps for storage. Grids may be stored for long periods of time in a tube. Our experience has been that they are safe for months. We think they will be secure for years and most likely decades.

Step 11-- When ready to examine grids place fine forceps in undercut notch of the GridStick™ and remove grids. You may reuse the GridStick™ at least six or more times.

Other Possible Uses For The GridStick™

- Special staining procedures
- Collecting serial sections
- Grid support for carbon coating
- Emulsion coating for autoradiography
- Critical point drying of whole mounts
- Preliminary check of formvar films on slot grids at the light level.
- Preliminary inspection of biological material at the light level using a phase microscope.

Ordering Information:

Product No.

155   SynapTek^{TM} GridStick^{TM} Kit, includes: 5 coated GridSticks, 10 Pipettes, 2 flow-limiting plugs

155-1 SynapTek^{TM} GridStick^{TM} Kit, pkg/5 kits

155-5 Replacement GridSticks, pkg/10

155-7 Replacement Pipettes, pkg/20

155-9 GridStick™ Adhesive, 5ml bottle