**Alexander Stain** **Protocol**

**Original protocol:**

Inflorescences are collected from adult plants and can be stored several days at room temperature in 10% ethanol or fixed in FPA50 for 1 to 3 hours. Anthers from mature flowers or buds of 1-2 mm are isolated and put on slides. A few drops of Alexander stain are added. A coverslip is put on and light hand pressure is exerted. Observation is made **15 min** later under a light microscope optionally equipped with differential interference contrast (DIC). The pollen wall is colored in green and cytoplasm of viable pollen grains in magenta-red-purple. Aborted pollen stain blue-green.

Alexander stain :

Ethanol 95% 10 ml

Malachite green (1% in 95% Ethanol) 1 ml

Fuchsin acid (1% in water) 5 ml

Orange G (1% in water) 0.5 ml

Phenol 5g

Glacial acetic acid 2 ml

Glycerol 25 ml

Distilled water 50 ml

For a working solution, dilute 1:50 in H2O. Note that the original recipe for this stain includes chloral hydrate (a controlled substance in the USA). For viability staining, chloral hydrate can be omitted.

**Sevan’s Protocol:**

1. Collect anthers from buds that are about to open.

2. Store anthers in 10% EtOH in 1.5 ml centrifuge tubes until use. If using anthers immediately, no need to add EtOH.

3. Add 150 μl\* Alexander stain (this is a lot, see notes below). Be careful to keep the tube as still as possible. You can leave the anthers in the stain at 4oC indefinitely if need be (this may work only if there is EtOH). Wait 15 min.

4. Place the contents of the tube on a slide and view using a light microscope. Red are viable and green are inviable.

**Notes:**

1. When you put the anthers in the tube and add the stain, the anthers will absorb the stain, and the supernatant will turn clear (so the anthers will be visible).

2. Some people fix anthers in a mixture of EtOH & acetic acid (3:1), and then store in EtOH.

3. Above it says to add the stain to anthers after placing them on slides and wait 15 min. Francesco needed to wait longer because he added the stain to tubes with EtOH.

4. \*You can add less stain, and let the anthers sit for longer (unexposed to light). I let them sit overnight when I want to conserve stain.

5. Viable pollen walls stain green but the protoplasm stains red if viable. Aborted grains have no protoplasm so they are just green.