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# Pollen Acetolysis V.2

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**We use this protocol and it's working**

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## Abstract

Custom pollen acetolysis procedure using a slightly modified version of standard acetolysis.

## Troubleshooting

## Sample Prep:

1 Anthers are placed into a 1.5mL tube and crushed.

2 Add glacial acetic acid and stir.

3 Centrifuge for 3min at 3700rpm to allow pollen to form a pellet at the bottom of the tube. If pollen sticks to the side of the tubes, rotate the tubes 180 degrees and re-centrifuge for 30s.

4 Draw out liquid with a pipette without disturbing the pellet. If pellet is disturbed, returnthe liquid to the tube, re-centrifuge and draw out again.

## Acetolysis:

5 Add 300uL 9:1 acetic anhydride: sulfuric acid acetolysis solution to samples and immediately transfer to heat block.

6 Incubate at 90°C for about 3-10min with caps open. Finished samples should appear brown in color

## Glacial Acetic Acid Wash:

7 Add 300uL of glacial acetic acid to all heated samples to stop the reaction.

8 Cap then vortex thoroughly. Rinse all glassware with glacial acetic acid and then water.

9 Spin at 3700 rpm for 3min

10 Draw out liquid using pipette.

11 Repeat glacial acetic acid wash one or two additional times

## Water Wash:

12 Add 300uL of water using a clean pipette tip.

13 Cap samples, vortex then spin again.

14 Draw water down to pollen pellet.

15 Repeat water wash one more time.

## Ethanol Wash:

16 Add 300uL of 50% ethanol to each sample.

17 Vortex thoroughly.

18 Spin at 3700rpm 3min.

19 Draw liquid out with pipette down to the pollen pellet.

20 Repeat procedure with 70% and 95% ethanol.

## Add Glycerin:

21 Add 3 drops of 1:1 glycerol/water to pollen residue.

22 Place on heat block at 25°C and leave overnight.

23 Mix samples, then mount on glass slide for viewing.

## Adopted From:

24 Erdtman G (1960) The acetolysis method- a revised description. Svensk Botanisk Tidskrift 54: 561–564.

25 Jones GD (2014) Pollen analyses for pollination research, acetolysis. Journal of Pollination Ecology 13: 203–217.

26 Park KB, Kim JS, Chung GY, Kim JH, Son DC, Jang CG (2022) Clematis pseudotubulosa (Ranunculaceae), a new species from Korea. Korean Journal of Plant Taxonomy 52: 35–44.

27 Pollen processing reference manual (2014) Florida Institute of Technology.