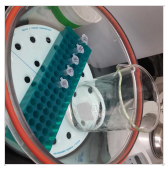

Sep 14, 2021

Sterilizing the Surface of Seeds by Chlorine Gas

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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ABSTRACT

This protocol sterilizes the surface of seeds for use in clean culture conditions without damaging the seeds germination or growth ability.

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MATERIALS TEXT

- Bleach, household, FisherSci NC9724348
- Hydrochloric acid, 37%, Acros AC450560050
- Beaker, 250 mL, FisherSci FB101250
- Desiccator, heavy glass, non-vacuuming, no desiccant, FisherSci 08-595E
- Microtube rack, FisherSci 22-313630
- 2 mL microcentrifuge tube
- Chemical Fume Hood
- 5 mL pipet, either a serological pipet with dispenser or a macro pipette tip with pipetter.
- Biological Safety Hood or Laminar Flow Hood

SAFETY WARNINGS

This protocol uses both chemical fume hoods and biological safety cabinets (laminar flow hoods). Understand the difference and how to safely and appropriately use both before performing the protocol.

This protocol intentionally produces chlorine gas which is toxic to human health. Please read all manufacturer safety data sheets before handling. UIUC personnel performing this protocol should be current on "Laboratory

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Safety", "Chemical Safety- An Introduction", and "Chemical Spills" Division of Research Safety training modules before performing this protocol.

Dispose of chemicals per your institutions hazard waste policy. For UIUC, solution can be neutralized to between pH 6 and 10 and discarded normally.

1 Move an aliquot of seeds to be sterilized to a 2 mL microcentrifuge tube. Label clearly.

2 Document the seed vials identifications and order via photograph or notetaking.

Exposure to chlorine gas can cause some types of ink, both marker and printed, to fade.

3 Place a desiccator in the chemical fume hood. Adjust the fume hood sash to a safe height.

4 Place the seed rack in a desiccator with no desiccant. Open the tube lids.

5 Fill a beaker with about **100 mL** of bleach, using graduations on the side of the beaker is sufficiently accurate for this purpose.

6 Place the beaker of bleach in the desiccator.

7

Quickly pipet **3 mL** of hydrochloric acid into the beaker of bleach.

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The reaction of bleach and hydrochloric acid will form chlorine gas. Do not breath in vapors. Ensure fume hood is in good operation. Wear appropriate PPE when handling hydrochloric acid.

8 Close the lid of the desiccator. If the desiccator contains any vacuum lines or vents, ensure that those valves are all in the closed position.

9 Allow the seeds to be exposed to the chlorine gas for a minimum of 3 hours.

10 Move the sealed desiccator to a biological safety cabinet that has been properly sterilized.

11 Open the desiccator slowly.

12 Close the seed vials. Seed vials can be stored outside the biological safety cabinet but should only be opened in properly sterilized biological safety cabinets or laminar flow hoods to ensure that they remain sterile.

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