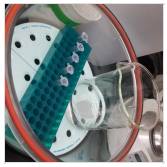


Version 2

Oct 04, 2021

Sterilizing the Surface of Seeds by Chlorine Gas V.2

Lynn Doran[1]

[1]Realizing Increased Photosynthetic Efficiency (RIPE)

This protocol is published without a DOI.

Burgess Lab UIUC

Lynn Doran
Realizing Increased Photosynthetic Efficiency (RIPE)

ABSTRACT

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

PROTOCOL CITATION

Lynn Doran 2021. Sterilizing the Surface of Seeds by Chlorine Gas. **protocols.io** https://protocols.io/view/sterilizing-the-surface-of-seeds-by-chlorine-gas-bynppvdn Version created by Lynn Doran

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Lindsey III, B. E., Rivero, L., Calhoun, C. S., Grotewold, E., Brkljacic, J. Standardized Method for High-throughput Sterilization of Arabidopsis Seeds. *J. Vis. Exp.* (128), e56587, doi:10.3791/56587 (2017).

CREATED

Sep 29, 2021

LAST MODIFIED

Oct 04, 2021

PROTOCOL INTEGER ID

53679

MATERIALS TEXT

- Bleach, household, FisherSci NC9724348
- Hydrochloric acid, 37%, Acros AC450560050
- Beaker, 250 mL, FisherSci FB101250
- Desiccator, Thermo Scientific Nalgene–Mfr # 5311-0250
- 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. 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 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 5 and 10 using 10M Sodium Hydroxide solution and discarded normally.

BEFORE STARTING

The volumes of reagents in this protocol were calculated for 6% chlorine gas in a 11,150 mL container (11" W X 13" H X 9" D).

If a different size container is used or a different percent gas is desired, adjust volumes of bleach and hydrochloric acid using the worksheet provided in Lindsey et al, 2017.

Worksheet for calculating chlorine gas concentration Lindsey et al. 2017.xlsx

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

If too many seeds are sterilized at once, seeds on the bottom layers may not be exposed to as much gas as top layers.

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

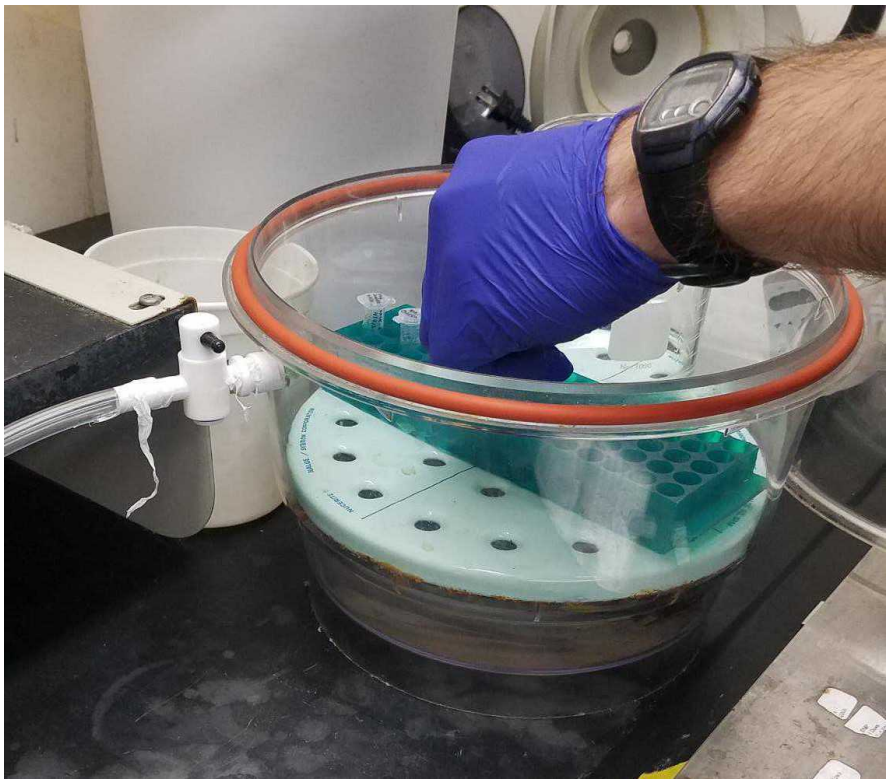
Exposure to chlorine gas can cause some types of ink, both marker and printed, to fade.

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 250 mL beaker with about **160 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 **5 mL** of hydrochloric acid into the beaker of bleach. Pipet all 5 mLs in one volume to avoid losing chlorine gas.

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.

Do not use a laminar flow hood. The biological safety cabinet will both keep the seeds sterile and protect you from any residual chlorine gas. A laminar flow hood will keep the seeds sterile but will blow residual chlorine gas back out at the operator.

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.