Jun 13, 2025 Version 1

Protocol for a Small-scale Algal Toxicity Assay for Testing the Acute Toxicity of Plastic Leachates V.1

DOI
https://dx.doi.org/10.17504/protocols.io.81wgbkjyygpk/v1

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Protocol Citation: Gillian Champoir 2025. Protocol for a Small-scale Algal Toxicity Assay for Testing the Acute Toxicity of Plastic Leachates. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbkjyygpk/v1

Protocol status: Working
We use this protocol and it's working

Created: June 13, 2025

Last Modified: June 13, 2025

Protocol Integer ID: 220157

Keywords: plastics, plastic research, plastic toxicity testing, algal assay, algal acute toxicity, plastic leachate, toxicity assay, scale algal toxicity assay, acute toxicity of plastic leachate, potential plastic contamination, acute toxicity, glass culture tube, assay, fluorescence, experiment, leachate, contamination

Funders Acknowledgements:
Ohio Sea Grant

Abstract

This protocol uses glass culture tubes as opposed to a well plate to avoid potential plastic contamination. The assay runs for two weeks or longer if so desired. Instead of using cell counts, fluorescence is used as a proxy for growth rate, as it can be calculated into μ upon completion of the experiment.

Materials

-tube rack
-10mL glass culture tubes
-glass serological pipettes
-micropipettes (P20, P200, P1000) and corresponding tips
-fluorometer
-plant growth chamber or grow light in a room with a constant temperature
-algal culture (of your choice; we used Scenedesmus dimorphus UTEX 1237 and Microcystis aeruginosa UTEX 2385)
-algal growth media (we used Bristol's Modified Media)
-plastic leachate (made in algal growth media)

Algal Assay for the Purpose of Acute Toxicity Testing of Plastic Leachates

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Note

We developed this protocol for our own plastic experiments that focus on plastic preproduction pellets. The pellets come small enough to be classified as microplastics (<5mm) without altering them in any way. Cutting up post-consumer environmental plastics, consumer plastics, etc. to various sizes as needed for experimentation is acceptable and will work with the protocol. It is recommended to take the average metrics (mass, volume, density, etc.) of the plastic pieces prior to use.

This protocol is also identical across plastic types (the 7 recycling categories used to distinguish between commonly used plastics).

Materials

2 -tube rack
-10mL glass culture tubes
-glass serological pipettes
-micropipettes (P20, P200, P1000) and corresponding tips
-fluorometer
-plant growth chamber or grow light in a room with a constant temperature
-algal culture
-algal growth media
-plastic leachate (made in algal growth media)

Preparation

3d

3 Have leachate pre-made in the freezer or start a fresh batch of leachate 72hrs before starting the assay. For a protocol on how to make plastic leachate, see:

Protocol

NAME

Protocol for Creating Plastic Leachates

CREATED BY
Gillian Champoir

Preview

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3.1

Note

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This protocol is also identical across plastic types (the 7 recycling categories used to distinguish between commonly used plastics).

3.2 -Glass receptacles with rubber stopped lids
-Balance Scale
-Benchtop shaker
-Thermometer
-Glass serological pipettes
-Metal mesh colander
-Vacuum filtration system
-Whatman GF/F glass fiber filters in **0.7µm**
-Freezer (-20°C)

3.3 Make leachates in glass bottles to avoid cross contamination. Fill the bottle with a set amount of solvent in mL. Solvent can be interchangeable depending on the experiment (synthetic freshwater/saltwater, algal media, filtered environmental water samples, etc).

3.3.1 Weigh your plastic material. Place a known weight (in milligrams) into a known amount of solvent (in milliliters). It is recommended to make a large batch of the highest

concentration (mg/ml) of leachate you intend to use. Using the original solvent, you can dilute the leachate to lower concentrations.

[EXAMPLE: Our experiment required concentrations of 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL, and 0.001 mg/mL
We made 100mLs of leachate at 1mg/mL and diluted small batches of it as needed.]

3.3.2 Place the bottle in a covered benchtop shaker (for incubation in the dark) and seal with cap. If room temperature is 25°C ± 2°C then the shaker can remain unheated; Otherwise use necessary means to achieve the temperature parameter. Set the RPM to 150.

3.3.3 150 rpm, 25°C, 72:00:00

3d

Leave incubating for 3 days.

3.3.4 Upon completion of incubation, leachates are filtered using vacuum filtration through a 0.7 µm Whatman Grade GF/F Filter to remove pellets and biotic contaminants. If the batch of leachate being made contains more than a few pellets, remove with the metal mesh colander prior to filtration.

3.3.5 Leachates are distributed into glass storage receptacles using 25mL borosilicate glass serological pipettes (to avoid plastic cross contamination) that have been previously acid-washed and autoclaved.

3.3.6 Take the pH of the leachates created. Leachates are either used immediately or stored frozen in glass receptacles at -20°C to prevent breakdown of compounds over time.

3.1 Assays are conducted in a series of 10 mL glass culture tubes, autoclave prior to use. Decide concentrations of leachates to be used.

[EX: We decided on five concentrations of plastic leachate tested in triplicate: 0 mg/mL, 1 mg/mL, 0.1 mg/mL, 0.01 mg/mL, 0.001 mg/mL]

3.2 Set up your tube rack prior to culturing. A standard autoclavable rack is 12×6. If you test in triplicate, you can set up 2 plastic types per rack, leaving gaps of 1 or 2 rows between each set of concentrations.

4 The culture of chosen algae or cyanobacteria should be started three days prior to using it for inoculation in the assay. It is important that the volume of the culture is large enough to be used for the assay without running out.

3d

[EX: we used Scenedesmus dimorphus UTEX 1237 cultured in Bristol's Modified Media (BMM) and Microcystis aeruginosa UTEX 2385 in 2N BG-11. Remember the leachate needs to be made in the same media that the culture requires.]

Protocol

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5 Total volume of each culture tube should be 5ml. The control tubes will be 4.75ml of culture media which is inoculated with 250 µL of culture, totaling 5ml.

5.1 **Total contents of each tube:**
1 mg/ml tubes: 250 µL of culture + 4.75ml of leachate
0.1 mg/ml tubes: 250 µL of culture + 475 µL of leachate + 4.275ml of culture media
0.01 mg/ml tubes: 250 µL of culture + 47.5 µL of leachate + 4.702.5ml of culture media
0.001 mg/ml tubes: 250 µL of culture + 4.75 µL of leachate + 4.745.25ml of culture media
0 mg/ml (Control) tubes: 250 µL of culture + 4.75ml of culture media

6 Fluorescence at time 0 hrs. should be measured for all tubes using a fluorometer. Decide to take measurements as either raw fluorescence or direct concentration- remain consistent through the sampling period.

[EX: we have a TD-700 fluorometer (Turner Designs) that only goes up to 1000rfu when measuring raw fluorescence, therefore we used direct concentration and calculated rfu from there.]

7 Samples are incubated together on a shaker table with agitation set to approximately 100RPM. In a Plant Growth Chamber, it is easy to set the temperature and light parameters to the optimal conditions for the species being used in your experiment. If you are incubating the samples outside of a chamber, ensure to take the temperature and light measurements of the room.

8 Fluorescence is then recorded every 24 hours for a period of two weeks, or until growth has plateaued, in an Excel spreadsheet.

Protocol references

Arensberg, P., Hemmingsen, V. H., & Nyholm, N. (1995). A miniscale algal toxicity test. Chemosphere, 30(11), 2103–2115. https://doi.org/10.1016/0045-6535(95)00164-1

OECD. (2011). Test No. 201: Freshwater alga and cyanobacteria, growth inhibition test. https://doi.org/10.1787/9789264069923-en