Buffers for Chloroplast Isolation from Diatoms

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Abstract

Guide for reagent/buffer preparation for protocol to separate chloroplasts from diatom cells using ammonium fluoride to permeate the silica frustrule and a percoll gradient to separate the plastid from other cellular components.

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Guidelines

Buffers and reagents can be mixed up ahead of time and stored cold (refrigerated). Percoll gradients (layering of 40% and 80% percoll solutions) should be done the same day of the extraction shortly before use.

Protocol

Isolation Buffer (40 mL)

Step 1.
Isolation Buffer (40 mL)
1) Mix:

- 20 ml 1 M sorbitol
- 400 µl 0.6 M Na_2-EDTA
- 200 µl 1 M $MgCl_2$
- 400 µl 1 M KCl
- 40 µl 1 M $MnCl_2$
- 2 ml 1 M HEPES-KOH pH = 8.0
- 16.96 ml Sterile H_2O.

2) Add bovine serum albumen (BSA) 1% (w/v) just before use to subset of isolation buffer needed at the beginning of the procedure

PEG-6000 Solution

Step 2.
PEG-6000 Solution
Mix:

- 6 g PEG

- 10 ml water

Percoll Solution (20 mL Stock)

Step 3.

Percoll Solution (20 mL Stock)

Mix:

- 19 ml Percoll
- 1 ml PEG – 6000 solution
- 0.2 g Ficoll
- 0.2 g BSA

Gradient Mixture (10 mL Stock)

Step 4.

Gradient Mixture (10 mL Stock)

Mix:

- 250 µl 1 M HEPES-KOH pH = 8.0
- 1 ml 0.1 M EDTA
- 6.26 ml 1 M sorbitol
- 2.49 ml water

3X Bottom Layer (80% Percoll)

Step 5.

3X Bottom Layer (80% Percoll)

Mix:

- 5.7 ml Percoll Solution
- 1.01 ml Gradient Mixture

3X Top Layer (40% Percoll)

Step 6.

3X Top Layer (40% Percoll)

Mix:

- 5.04 ml Percoll Solution
- 6.96 ml Gradient Mixture

10M Ammonium Fluoride (NH4F)

Step 7.

10M Ammonium Fluoride (NH_4F)

Mix:

- 3.7 g NH_4F
- 10 ml H_2O