TECHNICAL MANUAL

Chloroplast Isolation Kit

- **SKU CODE:** ARMA00350
- **SIZE:** 30 x 1 g Plant Tissue Extractions
- **DETECTION PRINCIPLE:** Density-Cushion Differential Centrifugation
- **RUO:** Research-Use-Only

Revised: 08/26

Chloroplast Isolation Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Intended Use

The Chloroplast Isolation Kit provides all of the buffers and reagents required for the rapid isolation of intact, functional chloroplasts from plant tissue. The kit contains sufficient material for the extraction of 30 x 1 g samples of plant tissue.

This manual must be read in its entirety before using this product. **For Research Use Only. Not to be used for diagnostic or therapeutic purposes.**

2. Introduction

Chloroplasts are specialised organelles of eukaryotic phototrophs that conduct photosynthesis through the structural compartmentalisation of light-capturing and carbon-assimilating machinery. Originating via endosymbiosis of an ancestral cyanobacterium by a non-photosynthetic eukaryote, chloroplasts retain a reduced circular genome (cpDNA), a prokaryotic 70S ribosome population, and a double-membrane envelope augmented in land plants and green algae by an internal thylakoid network.

Light absorption by chlorophyll and accessory pigments within photosystems II and I drives electron flow, splitting water at the oxygen-evolving complex and pumping protons into the thylakoid lumen. The resulting proton motive force powers ATP synthase, while reducing equivalents and ATP drive carbon reduction in the stroma. Beyond photosynthesis, chloroplasts serve as hubs for lipid biosynthesis, sulfur assimilation, and retrograde signalling that modulates nuclear gene expression.

3. Materials Provided

Component	Quantity	Cap / Label Colour
Chloroplast Isolation Buffer	180 ml	NM
Cushion Buffer	6 ml	NM
Storage Buffer	50 ml	NM
Optiprep Density Medium	10 ml	NM
Sodium Ascorbate	500 mg	Red

4. Storage and Handling

Store the unopened kit at 4°C. Keep all kit components on ice before and during the chloroplast isolation procedure.

5. Other Supplies Required

- 10–15 ml tissue homogeniser with a loose "large clearance" pestle, or a mortar and pestle (a bead beater may be used as an alternative).
- Miracloth or 100 µm nylon mesh.
- Razor blade or surgical scissors.
- 15 ml conical tubes and 1.5–2 ml Eppendorf-type tubes.
- Refrigerated centrifuge capable of 3,000 × g with a disengageable brake.
- Large-orifice pipette tips (or regular tips cut ~0.5 cm from the end).
- DMSO and 50% glycerol (for cryopreservation).
- 80% acetone (for chlorophyll a estimation).
- Controlled-rate freezing container, aluminium foil and ice.
- Spectrophotometer capable of reading at 652 nm.

6. Before You Begin

- Starch granules in tissues can damage thylakoid membranes during centrifugation steps. To avoid this, store plant materials in the dark and cold for at least 4 hours (12 to 24 hours is preferable).
- Chill all buffers and apparatus on ice before starting the isolation procedure. Everything should be held close to 4°C as much as possible.
- Ascorbate can be rapidly oxidised in solution, so it is supplied as a dry powder. Soon before the initial homogenisation step, determine how much Chloroplast Isolation Buffer (CIB) will be needed (~6.1 ml/sample), transfer it to a separate container and dissolve ascorbate at a concentration of 1 mg/ml of CIB.
- Prepare 0.5 ml of cushion solution for each sample to be prepared, containing 0.3 ml of Optiprep and 0.2 ml of Cushion Buffer. Transfer 0.5 ml to individual 1.5 – 2 ml Eppendorf tubes and store them on ice.
- During the procedure, work in subdued lighting when possible.

7. Isolation Procedure

Keep all buffers, apparatus and samples close to 4°C throughout the procedure and work in subdued lighting where possible.

1. Use a loose "large clearance" pestle in a 10-15 ml homogeniser or a mortar and pestle.
2. Remove any large veins from the sample tissue. Slice leaves into thin 1 mm strips using a razor blade or surgical scissors and transfer the strips (~1 g) to the homogeniser.
3. Add 4 ml of ice-cold CIB containing ascorbate to the homogeniser and gently grind the tissue using 4-6 up and down strokes total. Stop when the liquid looks an opaque uniform dark green.

   Note: If using a bead beater, freeze the tube holder in liquid N2 or a -80°C freezer for 15 minutes before loading the sample tubes. Chop tissue into 2-3 mm pieces then

add to the sample tube. Add 1.5 ml of ice-cold CIB then add the bead(s), close and run at 25-0-25 Hz for 3-5 seconds maximum. The sample should look crushed and shredded, not a smooth puree or paste. Open the tube and add 3.5 ml CIB to rinse the bead(s) and dilute the sample.

4. Moisten a piece of Miracloth or 100 µm nylon mesh with a few drops of CIB then filter the sample through the mesh into a 15 ml conical tube.
5. Rinse the homogeniser or bead beater with 1 ml of CIB and filter/combine with the previous portion of sample. Gently squeeze the Miracloth to recover adhering fluid. The volume of filtrate should be close to 5 ml.
6. Centrifuge at 200 × g for 3 minutes at 4°C. Decant the supernatant to a fresh tube and discard the pellet.
7. Centrifuge at 1500 × g for 5 minutes at 4°C. Carefully pour off the supernatant without disturbing the pellet.
8. Add 1 ml of ice-cold CIB to the pellet and resuspend it with slow gentle swirling. DO NOT vortex. Chloroplasts are extremely fragile.
9. Use a large orifice pipette tip, or cut a regular tip ~0.5 cm from the tip to create a large orifice, and transfer the chloroplast suspension to the Eppendorf tubes prepared earlier containing the cushion solution, layering it on top of the cushion without any mixing of the phases.
10. Centrifuge at 3000 × g for 10 minutes at 4°C with the centrifuge brake disabled. Intact chloroplasts will pass through the Optiprep cushion and form a pellet. Broken chloroplasts and other debris will remain floating on top of the cushion.
11. Carefully aspirate away the top layer and Optiprep cushion, leaving the pellet intact and undisturbed. Add 1.5 ml of Chloroplast Storage Buffer (CSB) and gently swirl to resuspend the pellet.
12. Centrifuge at 1000 × g for 5 minutes at 4°C and discard the supernatant.
13. Resuspend the pellet in a small volume (200 µl) of CSB, wrap in aluminium foil and store on ice in the dark until used.

Use the chloroplasts immediately or prepare for storage. Healthy isolated chloroplasts retain activity for 4-6 hours on ice in the dark. They will lose up to 40% of their photosynthetic activity overnight.

8. Storage of Isolated Chloroplasts

To stabilise for storage, add DMSO to 5% and glycerol to 10% (10 µl DMSO and 40 µl of 50% glycerol for a 200 µl chloroplast sample). Freeze to -80°C slowly, using a controlled-rate freezing container (plastic container filled with 100-200 ml 100% IPA) or a "CoolCell" container overnight. Once frozen, if available, quickly transfer samples from -80°C to liquid nitrogen.

To use, thaw rapidly, placing samples in a 25-30°C water bath until only a sliver of ice remains. Do not thaw on ice or at room temperature, since ice crystals can reform and damage chloroplast membranes. Dilute the samples into 10 volumes of CSB and centrifuge at 1000 × g for 5 minutes, then pour off the supernatant containing DMSO and glycerol. Resuspend in 200 µl of CSB. The chloroplasts are ready for use.

Long-term storage can cause loss of enzyme activity, but proper treatment should preserve most activity and maintain stable protein import, thylakoid stacking and robust electron transport for up to 6-10 months. You can expect an immediate 15-30% drop in activity from the initial freeze shock, but remaining activity should stabilise and not decrease further.

Storage at -80°C, maximum duration 1-2 months. At -80°C, ice recrystallisation occurs slowly over time, gradually micro-fracturing the membranes. Upon thawing: within 2 weeks, high preservation of function (80-90%) for robust processes like electron transport tracking (Hill reaction). At 1-2 months, metabolic functions like carbon assimilation (Rubisco) drop sharply (40-50%) due to stromal enzyme leakage. Beyond 2 months, thylakoid membranes uncouple; chloroplasts may still be used for DNA/RNA/protein extraction, but they are no longer viable for complex functional assays.

9. Chlorophyll a Estimation

Add 25 µl of sample to 475 µl of 80% acetone in an Eppendorf tube. Centrifuge at 3000 × g for 5 minutes. Read the OD at 652 nm.

Chlorophyll a concentration = OD652 nm / 1.8 mg/ml

10. Important Notes

- This kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
- Please read the instructions carefully before beginning the procedure. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
- Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
- If your tissue type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
- Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the procedure.

Notes:

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.