OrgFrontier™ Chloroplast Isolation Kit

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(Catalog # K468-10, 10 g Extractions; Store at -20°C)

I. Introduction:

Chloroplasts are membrane-bound organelles found in plant and algal cells which perform the function of photosynthesis. Plant chloroplasts are lens-shaped, usually 5-10 µm in diameter and 1-3 µm thick. Although leaves contain the majority of plants' chloroplasts, they can also be found in stems, seeds, and un-ripened fruit. BioVision's Chloroplast Isolation Kit provides an easy method to isolate chloroplast from a broad range of plant types including dicots, monocots, and conifers. Isolated chloroplasts can be used to study photosynthetic processes and as starting materials for chloroplast membrane, protein, chloroplast DNA and chloroplast RNA isolations.

II. Applications:

- Isolation of high purity chloroplasts from leaves/plant tissues
- Study photosynthetic processes, chloroplast membranes, thylakoid membranes, and associated proteins

III. Sample Type:

- Leaves, conifer needles, and other chloroplast-containing plant tissues

IV. Kit Contents:

Components	K468-10	Cap Code	Part Number
Chloroplast Isolation Buffer, 2X	500 ml	WM	K468-10-1
BSA (10% solution)	10 ml	WM	K468-10-2
DTT (1 M)	1 ml	Green	K468-10-3
Opti-Prep™ Density Gradient Medium	50 ml	NM	K468-10-4

V. User Supplied Reagents and Equipment:

- Blender, mortar and pestle, and/or homogenizer
- Bench-top or other centrifuge with variable speeds (up to 4000 x g) and controlled low-temperature capabilities that can be used with 15 and 50 ml tubes
- 15 and 50 ml centrifuge tubes
- Funnels & Collection vessels (beakers)
- Optional: sterile sand or metal beads, mortar and pestle
- Soft paint brush
- Miracloth, cheesecloth
- Pasteur pipettes

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Thaw the Opti-Prep™ Density Gradient Medium , BSA solution and the Chloroplast Isolation Buffer. Mix thoroughly before use.

- **Chloroplast Isolation Buffer**: Prepare 1X Chloroplast Isolation Buffer by adding equal volume of ddH_2O. Pre-Chill/keep on Ice reagents and materials for duration of chloroplast preparation.
- **Opti-Prep™ Density Gradient Medium Density Gradient Medium:** Upon receiving the kit, store undiluted reagent at 4 °C or RT. Bring to RT and mix well before use.

VII. Chloroplast Isolation Protocol:

1. **Sample Preparation:** Weigh out plant material (~10-20 g). Plant material must be cleaned. It can be washed and dried prior to storage. For best results, store plant material in the dark and cold (4 °C) for at least 4 hours. *Light exposure allows for the formation of starch granules which can rupture the chloroplasts during isolation*.
   - **a.** Prepare 14 ml of 1X Chloroplast Isolation Buffer per gram of plant material to be extracted: Prepare the required amount of 1X Chloroplast Isolation buffer by diluting the 2X buffer stock 1:1 with dH_2O water. Add 10 µl of the 10% BSA solution per ml of 1X Chloroplast Isolation Buffer. Just prior to use, add 1 µl of DTT per 1 ml of Chloroplast Isolation Buffer. *Label this buffer as 'Complete Buffer'*. Save a 10 ml aliquot, on ice, for the isolation procedure.
   - **b.** Chop or cut the plant material into small pieces (3 cm or less).
   - **c.** Add pieces to a blender or mortar and pestle, then add 4 ml of the Complete Buffer per gram of tissue. Thoroughly, blend (3-5 strokes) or grind the plant material. Avoid foaming.
     Note: Fine leaves can be ground or blended. Heavier leaves work better when blended. Very tough, fibrous leaves can be ground with sterile sand or metal beads and then blended or homogenized
   - **d.** Carefully pour the blended plant material through the 2-3 layers of cheesecloth in a funnel into a beaker. Then pour this filtrate through a second layer made of cheesecloth in a Miracloth-lined funnel into a second beaker. Squeeze cloth to remove remaining liquid. Make sure that there are no large pieces remaining. Re-filter if needed.
   - **e.** Pour the filtrate into pre-chilled 50 ml tubes. Use no more than 35 ml of filtrate per tube. Centrifuge the filtrate (200 x *g*; 15 min.)
   - **f.** Pour the supernatant into fresh, iced-cold 50 ml tubes. The pellets contain plant debris, nuclei and whole cells and can be discarded.
   - **g.** Centrifuge the supernatant at 1100 x *g* for 15 min. *The green pellet contains the chloroplasts.*
   - **h.** Discard the supernatant. Gently break up the pellet using a soft paintbrush or by very gentle taping with a finger.
   - **i.** Resuspend the pellet(s) in 1-2 ml of Complete Buffer by gently stirring with a soft paintbrush or by gently tapping the tube to obtain a homogeneous chloroplast suspension.

2. **Purification of Intact Chloroplasts:**
   - **a.** Mix 2 ml of Opti-Prep™ Density Gradient Medium with 0.5 ml of the prepared Complete Buffer in a pre-chilled 15 ml tube to make 2.5 ml of 80% Opti-Prep™ Density Gradient Medium.
   - **b.** Mix 1.75 ml of Opti-Prep™ Density Gradient Medium with 3.25 ml of the prepared Complete Buffer in a separate pre-chilled tube to get a 35 % Opti-Prep™ Density Gradient Medium solution. Mix well.
   - **c.** Carefully layer the 35% Opti-Prep™ Density Gradient Medium on top of the 80% layer in a centrifuge tube (Figure b). Layer the chloroplast suspension on top of the 35/80 % Opti-Prep™ Density Gradient Medium. Centrifuge at 3200 x *g* for 40 min. If the 35% layer appearance is cloudy showing several green streaks, you may centrifuge the tube for additional 20 min. intervals.
     Note: The broken chloroplasts will stay on the top of the 35% layer. The intact chloroplasts will form a band at the interface between the 35% and 80% layers. Any remaining debris will be in the pellet at the bottom of the tube.
3. **Wash step:**
   - **a.** Prepare 15 ml of 1X Chloroplast Isolation buffer with no additives.
   - **b.** Using a pasteur pipette, collect the band of intact chloroplasts at the interface and carefully resuspend it in 3X volumes of the 1X Chloroplast Isolation Buffer.
   - **c.** Centrifuge at 1750 x *g* for 3-6 min. and remove the supernatant. *The pellet contains clean, intact chloroplasts*.
   - **d.** The pellet can be resuspended in 0.5 ml of 1X Chloroplast Isolation Buffer.
     Notes:
     - For functional assays: chloroplast suspension should be used as soon as possible to avoid loss of activity.
     - For nucleic acid extractions: remove wash buffer (Steps 3a – 3c) and omit Step 3-d. Intact, washed chloroplast pellets should be stored at -80 °C).
4. **Estimation of Chlorophyll a (if desired):**
   - **a.** Prepare 0.475 ml of an 80% acetone solution into an Eppendorf tube
   - **b.** Add 25 µl of the chloroplast suspension, mix well.
   - **c.** Centrifuge for 5 minutes at 3000 x g.
   - **d.** Read the absorbance at 652 nm using a Spectrophotometer. *Use 80% acetone solution as a blank*.
   - **e.** Calculate Chlorophyll a concentration using the formula:

$$Chlorophyll\ a\ concentration = \frac{OD_{652\,nm} * 20}{36} = \frac{OD_{652\,nm}}{1.8}\left(\frac{mg}{ml}\right)$$

Where= **20**: Dilution Factor
36: Extinction Coefficient (in ml/(cm·mg))

Chlorophyll concentration can be expressed in mg Chlorophyll a/mg protein

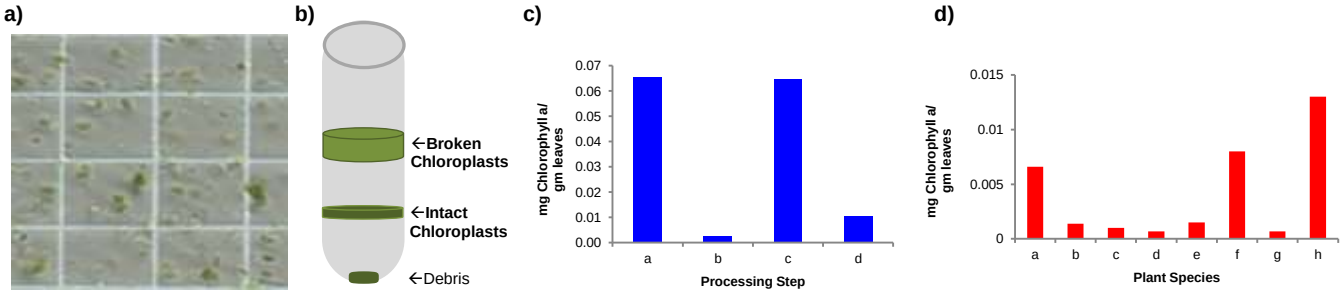

Figure: (a) Light microscope image of Chloroplasts Isolated from *Ficus* leaves; **(b)** Representation of a Chloroplast Gradient isolation of intact chloroplasts; **(c)** Chlorophyll *a* content at each processing step: *a*: debris pellet, first centrifugation (Step 1-f); *b*: supernatant from chloroplast (Step 1-g); *c*: Intact chloroplast layer (Step 2-c); *d*: broken chloroplast layer (Step 2-c); **(d)** Chlorophyll a content of isolated chloroplasts from multiple species: *a*: Spider Plant (*Chlorophytum comosum*); *b*: Corn husks (*Zea mays*); *c*: Pine needles (*Pinus muricata*); *d*: Cypress needles (*Cupressus sempervirens*); *e*: Juniper (*Juniperus species*); *f*: Lettuce (*Lactuca sativa*); *g*: *Stapelia variegata*; *h*: *Ficus species*.

VIII. RELATED PRODUCTS

Plant Tissue Extraction Kit (K296-50)
Plant Advance™ PCR Kit (M1144-25)
Lysosome Isolation Kit (K235-50)
Mitochondria Isolation Kit for Tissues & Cultured Cells (K288-50)
Plant Tissue Genomic DNA Isolation Kit (K316-100)
Yeast Mitochondria Isolation Kit (K259-50)
Yeast Nuclei Isolation Kit (K289-50)

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