Cyclohexane Diamine Tetraacetic Acid (CDTA) Extraction of Plant Cell Wall Pectin

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[Abstract] The goal of this procedure is to extract pectin from plant cell walls. Pectins are galacturonic acid containing polymeric sugars that are important components of plant cell walls. Various procedures aimed at studying plant cell wall components require the extraction of pectin. Pectin is synthesized in the Golgi apparatus in a highly esterified fashion and is de-esterified in the cell wall (Mohnen, 2008). Pectin is generally water soluble. De-esterified pectin can form so-called “egg-box structures” in the presence of Ca^{2+} ions (Mohnen, 2008; Harholt *et al.*, 2010). Pectin in these “egg-box structures” is cross-linked and less soluble. Cyclohexane diamine tetraacetic acid (CDTA) chelates Ca^{2+} ions and hence allows extraction of Ca^{2+} cross-linked pectin from cell walls.

Materials and Reagents

1. Plant material or cell wall preparation of choice (see Note 1)
2. Cyclohexane diamine tetraacetic acid (CDTA) (Fluka, catalog number: 34588)
3. Tris-base (Sigma-Aldrich, catalog number: T6066)
4. Sodium hydroxide (Thermo Fisher Scientific, catalog number: S318)
5. Liquid nitrogen
6. CDTA extraction buffer (see Recipes)

Equipment

1. Liquid nitrogen container
2. Paint shaker (*e.g.* Harbil, model: 5G-HD) and ball bearings (*e.g.* 3 mm diameter steel beads) (mortar and pestle or bead beater)
3. Media bottle
4. Single-channel pipettor
5. Microfuge tubes appropriate for use at 95 °C and for freezing in liquid N_2 (Thermo Fisher Scientific, catalog number: 02-707-355)
   Note: We use 2 ml screw cap tubes.
6. Pipette tips

7. Water bath or incubator
8. Vortex mixer
9. Microcentrifuge capable of spinning at 10,000 *x g*
10. Optional: Screw cap tubes or lid locks

Procedure

1. Pre-heat water bath or incubator to 95 °C and pre-heat CDTA extraction buffer.
2. Collect plant tissue in tubes - we use leaf material from approx. 4 week old *Arabidopsis thaliana* plants. Three fully expanded *Arabidopsis* leaves weigh about 250 mg.
3. Freeze tubes containing the tissue in liquid nitrogen (tissue can be stored at -80 °C).
4. Pulverize frozen tissue using ball bearings and a paint shaker, mortar and pestle or a bead beater. The method used to pulverize tissue is not critical. Keep tissue frozen. If paint shaker is used three cycles of 3 min each are generally sufficient to pulverize tissue.
5. Add 1 ml of CDTA extraction buffer to 250 mg of frozen pulverized *Arabidopsis* tissue.
6. Place tubes at 95 °C. Use caution, the liquid will become very hot and lids may open; therefore use screw cap tubes or lid locks to secure lids.
7. Incubate for 15 min, vortex every 5 min.
8. Centrifuge at 10,000 *x g* for 10 min at room temperature.
9. The supernatant contains the pectin.
10. Supernatant can be frozen and freeze dried for downstream applications.

Notes

1. We directly use pulverized *Arabidopsis* leaf material (Bethke *et al.*, 2014). Alternatively crude cell wall preparations like alcohol insoluble residue (Gille *et al.*, 2009) preparations *etc.* can be used (pre-heat the water bath and extraction buffer and proceed to step 5).
2. Different groups have reported different extraction times and temperatures *e.g.* 15 min at 95 °C (Siedlecka *et al.*, 2008) or 4 h at room temperature (Moller *et al.*, 2008). In our experience about twice the amount of pectin could be extracted when extraction was performed for 15 min at 95 °C as compared to the longer extraction at room temperature.
3. Different groups have utilized similar protocols for the extraction of pectin from various monocotyledonous and dicotyledonous plants *e.g.* cucumber, tomato, celery (Jarvis *et al.*, 1982), poplar (Siedlecka *et al.*, 2008), soybean (Huisman *et al.*, 2001) or wheat (Wiethölter *et al.*, 2003).
4. The extraction buffer can be stored at room temperature for several weeks.

5. Pectin extracted using this procedure can be used for various downstream application including dot-blot analysis with pectin specific antibodies, sugar analysis, ion-exchange chromatography or determination of degree of esterification of pectin (Bethke *et al.*, 2014; MacDougall *et al.*, 1997).

Recipes

1. CDTA extraction buffer
   50 mM CDTA
   50 mM Tris-base
   Adjust pH to 7.2 using sodium hydroxide

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Many labs have used similar protocols in the past. We have adapted this protocol from Siedlecka *et al.* (2008).

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