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In Gel Detection of Lipase Activity in Crude Plant Extracts (*Olea europaea*)

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[Abstract] Here, we provide a detailed protocol describing a SDS-PAGE based procedure to assay in gel neutral lipase activity. Total protein extracts are separated by SDS-PAGE and gels are treated with lipase substrate-α-naphthyl palmitate. This long-chain fatty acid ester is hydrolysed by lipases present in the gel. The product resulting from this reaction can be then visualized in the gel as yellow-brownish activity bands. This relatively simple and effective method of lipase assay detection can be used for crude protein extracts from different plant tissues.

Materials and Reagents

1. Homogenized plant tissues
2. Liquid nitrogen
3. 30% acrylamide stock (29: 1 acrylamide: bisacrylamide) (Bio-Rad Laboratories, catalog number: 161-0156)
4. TEMED (Bio-Rad Laboratories, catalog number: 161-0801)
5. Ammonium persulfate (Sigma-Aldrich, catalog number: A3678)
6. 2D Quant Kit (GE Healthcare, catalog number: 80-6483-56)
7. Trizma® base (Sigma-Aldrich, catalog number: T1503)
8. Bromphenol blue (Sigma-Aldrich, catalog number: B0126)
9. Glycerol
10. Triton X-100 (Sigma-Aldrich, catalog number: T8532)
11. Na_2HPO_4 (Sigma-Aldrich, catalog number: S3264)
12. NaH_2PO4 (Sigma-Aldrich, catalog number: 71505)
13. *N,N*-Dimethylformamide (Fluka, catalog number: 72438)
14. Fast blue B salt (Sigma-Aldrich, catalog number: D9805)
15. α-naphthyl palmitate (Sigma-Aldrich, catalog number: N9875)
    Note: α-naphthyl palmitate catalog number: N9875 is no longer available in Sigma-Aldrich, but is available in Santa Cruz Biotechnology, catalog number: CAS 15806-43-6.
16. Extraction buffer (see Recipes)
17. 12% separating gel (see Recipes)
18. 4% stacking gel (see Recipes)

19. 2 x SDS sample buffer (see Recipes)
20. 1 M sodium phosphate buffer (see Recipes)
21. Developing solution (see Recipes)

Equipment

1. Mortar and pestle
2. Refrigerated centrifuge 5810R (Eppendorf, catalog number: 5810000.017) or similar, equipped with a rotor for 2 ml microcentrifuge tubes
3. Magnetic stirrer
4. 2 ml microcentrifuge tube
5. Tubular glass vials
6. Mini-PROTEAN® Tetra Cell (Bio-Rad Laboratories, catalog number: 165-8000)

Software

1. Quantity One software (Bio-Rad Laboratories)

Procedure

A. Tissue homogenization and protein extraction

Note: Do not denature the sample at any time. Proteins must remain in their native, folded state.

1. Homogenize tissue (0.2 g) to a very fine powder in liquid nitrogen using a precooled mortar and pestle.
2. Transfer the powder to a 2 ml microcentrifuge tube and add 1.5 ml of extraction buffer and mix well using vortex (see Note 3).

   Note: If samples are used immediately or stored properly it is not necessary to use protease inhibitors.
3. Stir sample in tubular glass vials using a magnetic stirrer for 1 h at 4 °C.
4. Centrifuge at 13,500 x *g* for 30 min at 4 °C.
5. After centrifugation, collect the supernatant.
6. Quantify extract and make aliquots.

   Note: The protein concentration was measured using the 2D Quant Kit following the manufacturer's instructions.

B. SDS-PAGE

1. Mix protein sample (approx. 25 µg per sample) with an equal volume of 2x SDS sample buffer. Do not heat at any time.
2. Load protein sample and perform the SDS-PAGE on a 12% separation gel.

C. In-gel detection of lipase activity

1. Remove SDS from the polyacrylamide gels by washing them three times for 30 min each in a solution containing 0.05 M phosphate buffer (pH 7.0) and 2.5 % (v/v) Triton X-100.
2. Incubate the gels for 30 min at 37 °C in a developing solution in the dark.
3. Wash gels with distilled water three times for 10 min on a shaker.
4. The gels can be stored in distilled water up to two months at 4 °C without a significant loss of staining intensity.
5. To quantify the level of lipase activity, the Quantity One software can be used to measure the density of appearing brown bands.

Recipes

1. Extraction buffer
   0.5 M sodium phosphate buffer (pH 7.0)
2. 12% separating gel
   Add the following solutions (total volume: 10 ml)

30% acrylamide/bisacrylamide	3 ml
1.5 M Tris-HCl (pH 8.8)	2.5 ml
10% SDS	100 µl
dH_2O	4.4 ml
10% ammonium persulfate	50 µl
TEMED	10 µl

3. 4% stacking gel
   Add the following solutions (total volume: 5 ml)

30% acrylamide/bisacrylamide	0.5 ml
0.5 M Tris-HCl (pH 6.8)	1.25 ml
10% SDS	50 µl
dH_2O	3.2 ml
10% ammonium persulfate	25 µl
TEMED	5 µl

4. 2 x SDS sample buffer
   100 mM Tris-HCl (pH 6.8)
   4% (w/v) SDS
   0.2% (w/v) bromophenol blue
   20% (v/v) glycerol
5. 1 M sodium phosphate buffer (pH 7.0)
   57.7 ml 1 M Na_2HPO_4
   42.3 ml 1 M NaH_2PO4
6. Developing solution

Mix 40 mg of α-naphthyl palmitate, prepared in 16 ml of *N, N*-Dimethylformamide with 80 mg of Fast blue BB salt in 144 ml of 0.1 M phosphate buffer (pH 7.0)

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