Expression, Purification and Enzymatic Assay of Plant Histone Deacetylases

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[Abstract] Histone deacetylases (HDACs) catalyzing the removal of acetyl groups from lysine residues of histone and non-histone proteins play vital roles in regulation of gene transcription. In plants, HDACs can be grouped into three families, including RPD3-type, SIR2-type and plant specific HD2-type HDACs. Here we describe a method to determine plant HDAC enzymatic activity. This protocol includes expression, purification and enzymatic activity assay of recombinant plant HDACs expressed in *Escherichia coli* (*E. coli*) and *Arabidopsis thaliana* (*A. thaliana*).

Materials and Reagents

1. Sterile Syringe Filters (Merck Millipore Corporation, Millex, catalog number: SLGV033RS)
2. Ice
3. Seeds of *A. thaliana* ecotype Columbia (Col-0)
4. *Escherichia coli* (BL21) (Thermo Fisher Scientific, Invitrogen™, catalog number: C6000-03)
5. pGEX-4T-3 expression vector (GE Healthcare, Amersham, catalog number: 28-9545-52)
6. Agrobacterium (GV3101)
7. LB medium (Caisson Laboratories, catalog number: LBP01-500 GM)
8. Murashige and Skoog (MS) media (Sigma-Aldrich, catalog number: M5524)
9. Ampicillin (Beyotime, catalog number: ST007)
10. Isopropyl-b-D-thiogalactopyranoside (IPTG) (Sigma-Aldrich, catalog number: 367-93-1)
11. Sucrose (Sigma-Aldrich, catalog number: 57-50-1)
12. Potassium hydroxide (KOH) (Sigma-Aldrich, catalog number: 1310-58-3)
13. Bacto Agar (Sigma-Aldrich, catalog number: 9002-18-0)
14. Ethanol
15. NP40 (Sigma-Aldrich, Abcam, catalog number: 9016-45-9)
16. Sodium chloride (NaCl) (Sigma-Aldrich, catalog number: 31434)
17. Potassium chloride (KCl) (Sigma-Aldrich, catalog number: 7447-40-7)

18. Sodium phosphate dibasic (Na_2HPO_4) (Sigma-Aldrich, catalog number: 7558-79-4)
19. Potassium phosphate monobasic (KH_2PO_4) (Sigma-Aldrich, catalog number: 7778-77-0)
20. Hydrochloric acid (HCl)
21. Ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich, catalog number: 60-00-4)
22. Glycerol (Sigma-Aldrich, catalog number: 56-81-5)
23. L-Glutathione reduced (Sigma-Aldrich, Abcam, catalog number: 70-18-8)
24. Ultrapure water
25. Liquid N_2
26. GST·Bind™ Resin (Merck Millipore Corporation, Novagen, catalog number: 70541)
27. Protease inhibitors (Roche Diagnostics, catalog number: 11873580001)
28. Glycine (Sigma-Aldrich, catalog number: 15527)
29. Tris base (Sigma-Aldrich, catalog number: 77-86-1)
30. GFP-Trap (ChromoTek, GFP-Trap®_M)
31. HDAC activity colorimetric assay kit (BioVision, catalog number: K331-100)
32. HeLa nuclear extracts (Biovision, catalog number: 1641-1)
33. Trichostatin A (Sigma-Aldrich, catalog number: 58880-19-6)
34. Bradford Protein Assay Kit (Beyotime, catalog number: P0006)
35. Bovine Serum Albumin (BSA) (Sigma-Aldrich, catalog number: A7906)
36. Phenylmethanesulfonyl fluoride (PMSF) (Sigma-Aldrich, catalog number: P7626)
37. Boc-Lys(Ac)-pNA
38. Phosphate buffered saline (PBS) (see Recipes)
39. GST Elution buffer (see Recipes)
40. Plant protein extraction buffer (see Recipes)
41. GFP Wash buffer (see Recipes)
42. GFP Elution buffer (see Recipes)
43. HDAC Assay Buffer (Biovision) (see Recipes)
44. HDAC substrate (colorimetric substrate) (see Recipes)

Equipment

1. Plant growth chamber (Panasonic Healthcare Corporation, model: MLR-352)
2. Sterile fume hood (Alibab, Airtech, model: VS-1300L)
3. Autoclave (HIRAYAMA, model: HVE-50)
4. Centrifuge (Eppendorf AG, model: 5418R and 5810R)
5. Shaker (Eppendorf AG, model: New Brunswick™ Innova® 40)
6. Sonicator (SONICS & MATERIALS, model: VCX130)
7. Spectrophotometer (Molecular Devices Spectra Max)

Procedure

A. Expression and purification of recombinant HDACs in *E. coli*

1. Insert the protein coding sequence of HDACs (*e.g.* HDA5) into the vector pGEX-4T-3 as described in Luo *et al.* (2015).
2. Transform the plasmid into *E. coli* (BL21) and select on fresh agar plates containing ampicillin (100 μg/ml).
3. Inoculate a single colony of *E. coli* into 2 ml LB media with rotation overnight at 37 °C.
4. Transfer 2 ml *E. coli* culture into 250 ml LB media with vigorous aeration at 37 °C and culture until OD_{600} reached 0.4-0.6.
5. Add a final concentration of 0.1 mM isopropyl-b-D-thiogalactopyranoside (IPTG) into bacteria solution and incubate with vigorous aeration about 6-10 h at 28 °C.
6. Centrifuge the cells at 6,000 *x g* for 10 min at 4 °C and remove the supernatant. Then resuspend the pellet with 25 ml of cell lysis buffer (1x PBS).
7. Sonicate the resuspended solution for 15 sec with 40% power for 40 times. Between each sonication treatment, place the solution on ice for 1 min.
8. After sonication, apply the soluble cell extract to GST affinity column.
9. Wash unbound proteins from the resin by adding 5 ml x 3 of wash buffer (1x PBS).
10. Elute the bound protein from the resin by adding 4 ml elution buffer.
11. Store the eluted protein at -20 °C.

B. Expression and purification of HDAC in transgenic plants

1. Clone HDAC cDNA (*e.g.* HDA5) into the pK7WGFP binary vector.
2. Transform the resultant plasmid into GV3101 Agrobacterium strain by electroporation.
3. Introduce the transformed Agrobacterium into *Arabidopsis* plants by floral dip (Zhang, *et al.*, 2006).
4. Select putative transformants on ½ MS media containing kanamycin (50 mg/L).
5. After screening, extract total soluble proteins by plant protein extraction buffer from transgenic plants, then centrifuge at 4 °C and 13,000 *x g* for 10 min.
6. Transfer the suspension to a fresh Eppendorf tube and centrifuge again at 4 °C and 13,000 *x g* for 10 min.
7. Purify GFP-HDAC recombinant protein by GFP-Trap according to the manufacturer's instructions. Add 25 μl bead slurry into soluble protein extract.
8. Tumble-end-over-end for 1 h at 4 °C.
9. Magnetically separate beads until supernatant is clear. Discard supernatant and repeat wash twice.
10. Elute bound proteins by adding 50 μl, 0.2 M glycine (pH 2.5).
11. Transfer the supernatant to a fresh Eppendorf tube and add 5 μl 1 M Tris base (pH 10.4) for neutralization.

12. Determine the protein concentration of the purified protein by Bradford assay approach (He, 2011).

C. HDAC enzymatic activity assay
   1. Place 85 µl purified proteins in the 96-well plate, then add 10 µl of 10x HDAC assay buffer and 5 µl of colorimetric substrate to each well and incubate at 37 °C for 1 h. HeLa nuclear extracts (4 µg) were used as positive controls. The HDAC inhibitor TSA was used to demonstrate the specificity of deacetylation activities.
   2. Stop the reaction by adding 10 µl of Lys developer and incubate the plate at 37 °C for 30 min.
   3. Measure the HDAC activity spectrophotometrically at 405 nm.

Representative data

For representative data, please see the papers of Luo *et al.* (2015).

Recipes

1. Phosphate buffered saline (PBS) (sterile filtered)
   10 mM Na_2HPO_4
   2 mM KH_2PO_4
   137 mM NaCl
   2.7 mM KCl
   pH 7.4
2. GST elution buffer (sterile filtered)
   50 mM Tris-HCl
   10 mM reduced glutathione
   pH 8.0
3. Plant protein extraction buffer (sterile filtered)
   10 mM Tris/Cl (pH 7.5)
   150 mM NaCl
   0.5 mM EDTA
   0.5% NP40
4. GFP wash buffer (sterile filtered)
   10 mM Tris/Cl (pH 7.5)
   50 mM NaCl
   0.5 mM EDTA
5. GFP elution buffer (sterile filtered)
   200 mM glycine (pH 2.5)
6. HDAC assay buffer

15 mM Tris-HCl (pH 8)
250 μM EDTA
250 μM NaCl
10% glycerol

7. HDAC substrate
   10 mM Boc-Lys(Ac)-pNA

Acknowledgment

This work was supported by the National Natural Science Foundation of China (31200965) and the China Postdoctoral Science Foundation (2014M562220). The histone deacetylase activity assay was modified from instruction of Biovision HDAC activity colorimetric assay kit.

References

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