Monolith Protocol MO-P-056

ALDH21 – $NADP^+$

Aldehyde dehydrogenases constitute a superfamily of NAD(P)+-dependent enzymes, which detoxify aldehydes produced in various metabolic pathways. Plants comprise 13 ALDH families, five of them, ALDH11, ALDH19, ALH21, ALDH22 and ALDH23, unique to plants. ALDH21 from the moss *P. patens* codes for a tetrameric $NADP^+$-dependent succinic semialdehyde dehydrogenase (SSALDH), which converts succinic semialdehyde, an intermediate of the c-aminobutyric acid (GABA) shunt pathway, into succinate in the cytosol. Lower plant species including some green algae, non-vascular plants (bryophytes) as well as the oldest vascular plants (lycopods) and ferns (monilophytes) possess a unique aldehyde dehydrogenase (ALDH) gene named ALDH21. However, the gene is absent in flowering plants. The expression of ALDH21 gene is strongly induced by a desiccation and turns back upon rehydration in the twisted mosses *S. ruralis* and *S. caninervis*. Also, the overexpression of ALDH21 in cotton and tobacco improves their drought and salt tolerance.

protein – small molecule interaction | plant biology | ALDH

A1. Target/Fluorescent Molecule

ALDH21
uniprot.org/uniprot/A9SS48

A2. Molecule Class/Organism

Cytosolic enzyme
Physcomitrella patens

A3. Sequence/Formula

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MTLGHMVQKA KESSGDVTPK KYNIFLASKP VDGDRKWLDV TNKYTNDVAA KVPQATHKDI DDAIDAAVAA APAMAAMGAY
ERKAVLEKVV AELKNRFEEI AQTLTMESGK PIKDARGEVT RTIDTFQVAA EESVRIYGEH IPLDISARNK GLQGIVKKFP
IGPVSMVSPW NFPLNLVAHK VAPAIAVGCP FVLKPASRTP LSALILGEIL HKIEELPLGA FSILPVSRED ADMFTVDERF
KLLTFTGSGP IGWDMKARAG KKKVVMELGG NAPCIVDDYV PDLDYTIQRL INGGFYQGGQ SCIHMQRLYV HERLYDEVKE
GFVAAVKKLK MGNPFEEDTY LGPMISESAA KGIEDWVKEA VAKGGKLLTG GNRKGAFIEP TVIEDVPIEA NARKEEIFGP
VVLLYKYSDF KEAVKECNNT HYGLQSGIFT KDLNKAFYAF EHMEVGGVIL NDSPALRVDS QPYGGLKDSG IQREGVKYAM
DDMLETKVLV MRNVGTL
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A4. Purification Strategy/Source

Total RNA was isolated from Physcomitrella ('Gransden 2004' strain) at the protonema stage using the RNAqueous kit and plant RNA isolation aid and treated twice with the Turbo DNase-free kit (Thermo Fisher Scientific). The cDNA was synthesized using Superscript III RT (Thermo Fisher Scientific) and oligo dT primers. The sequence coding for PpALDH21 (1494 bp) was amplified using gene-specific primers with SacI and KpnI sites (5'-CAAGAGCTCAATGACGCTCGGCCATAT-3', 5'-ATCGGTACCTTACAAGGTTCCAACATTCC-3') and the Accuprime Pfx polymerase (Thermo Fisher Scientific). The gene was further cloned into a pCDFDuet vector from Merck and then transformed into T7 express *E. coli* cells (New England Biolabs). Protein production was carried out at 20°C overnight. ALDH21 was purified on a HisPur Cobalt Spin Column (Thermo Fisher Scientific) and further on a HiLoad 26/60 Superdex 200 column (GE Healthcare scientific) into 20 mM Tris–HCl buffer, pH 8.0, 100 mM NaCl and 2% (w/v) glycerol. Protein content was measured using the Coomassie plus (Bradford) protein assay kit with bovine serum albumin (BSA) as a standard (Thermo Fisher Scientific).

A5. Stock Concentration/Stock Buffer

33 mg/mL | 583 µM (calculated for monomer) or 146 µM (calculated for the whole tetramer)
20 mM Tris–HCl buffer, pH 8.0, 100 mM NaCl, 4% (w/v) glycerol

A6. Molecular Weight/Extinction Coefficient

55.56 kDa (for 6xHis-tagged monomer)
46,090 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20, supplemented with 1 mg/mL BSA

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* 10 µg Dye RED-NHS | 1* B-Column

A9. Labeling Procedure

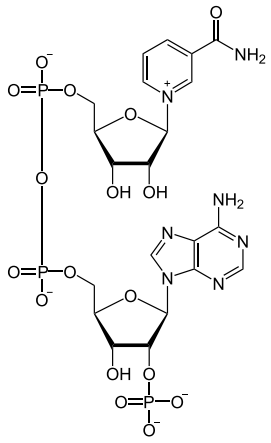
1. Add 95 µL of Labeling Buffer NHS to 5 µL of 200 µM calculated as monomer PpALDH21 to obtain 100 µL of a 20 µM solution.
2. Add 30 µL of DMSO to 10 µg Dye RED-NHS to obtain a ~470 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 12.5 µL of the 470 µM dye solution with 87.5 µL of Labeling Buffer NHS to obtain 100 µL of a 60 µM dye solution (3x protein concentration).
4. Mix PpALDH21 and dye in a 1:1 volume ratio (200 µL final volume, 6.25% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 300 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 600 µL of dilution buffer and collect the eluate.
11. Keep the labeled PpALDH21 (~3.3 µM) on ice in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

$NADP^+$ (β-Nicotinamide Adenine Dinucleotide)



B2. Molecule Class/Organism

Small organic coenzyme

B3. Sequence/Formula

$C_{21}H_{27}N_7NaO_{17}P_3$ (sodium salt)

B4. Purification Strategy/Source

Applichem Pancreac
A1394,0001

B5. Stock Concentration/Stock Buffer

30.6 mg/mL | 40 mM
50 mM Tris-HCl buffer substance at pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$

B6. Molecular Weight/Extinction Coefficient

765.39 Da

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 40 mM $NADP^+$ solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
2. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
3. Mix 2.5µL of labeled PpALDH21 (~3.3 µM) with 197.5 µL of dilution buffer to obtain 200 µL of ~40 nM PpALDH21. Add 10 µL of this solution to each tube from **16** to **1** and mix by pipetting.
4. Incubate tubes for 10 minutes at room temperature in the dark before loading capillaries.

D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

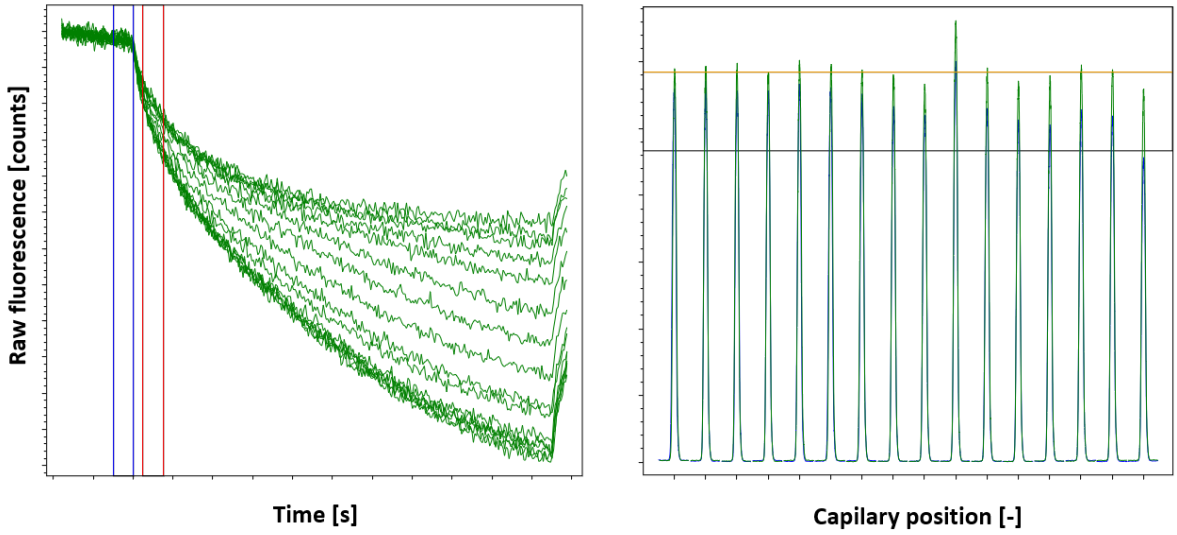
MO.Control v1.5 (NanoTemper Technologies GmbH)
MO.AffinityAnalysis v2.1 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

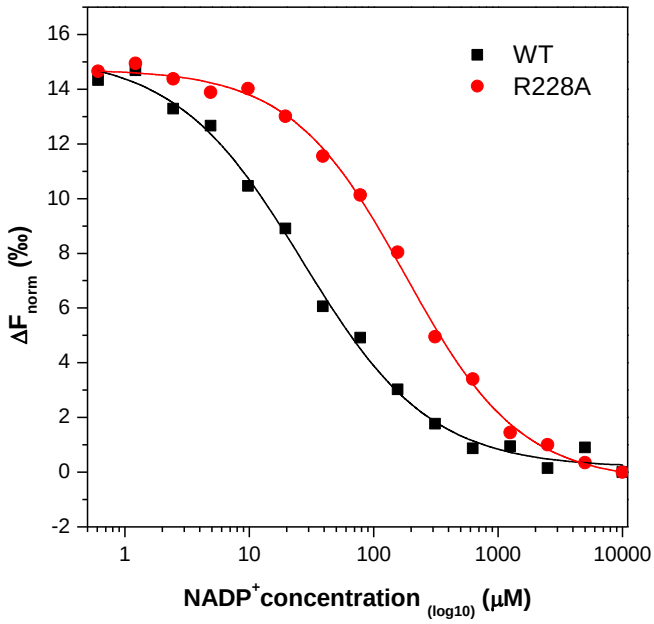
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20, supplemented with 1 mg/mL BSA
20 nM ALDH21 | 20 mM – 0.61 µM $NADP^+$ | 25°C | high MST power | 20% excitation power

D4. MST Results (Capillary Scan/Time Traces/Dose Response)

K_d = 31 ± 8 µM (*wt* PpALDH21)
K_d = 282 ± 50 µM (R228A mutant of PpALDH21)
Kopečný et al. (2017) Plant J. 92, 229-243





D5. Reference Results/Supporting Results

K_m = 24 ± 1 µM (*wt* PpALDH21)
K_m = 375 ± 12 µM (R228A mutant of PpALDH21)

Kinetically measured K_m values for the coenzyme $NADP^+$
Kopečný et al. (2017) Plant J. 92, 229-243

E. Contributors

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