DMADP assay

Wednesday, November 02, 2011
4:23 PM

2011-8-30
Henrys co...

Protocol for measuring DMADP in *E. coli*

Prepared by Thomas D. Sharkey July 22, 2011 with modifications by Sean Weise Nov 1, 2011
Based on (Brüggemann and Schnitzler, 2002; Copolovici and Niinemets, 2005; Henneman et al., 2011)

Summary: *E. coli* cells are harvested by centrifugation, broken open, extracted and deproteinized with acetonitrile. The extracts are centrifuged and the supernatants are transferred and dried in a speed-vac. The residue is resuspended in phosphoric acid, added to a sealed vial, incubated at 70°C, and the isoprene in the head space is taken by syringe for analysis in a GC with PhotoIonization Detector or Fast Isoprene Sensor. Alternatively the sample could be redissolved in HPLC solvent and analyzed by LC MSMS.

1. All steps are carried out on ice or as cold as possible to minimize spontaneous conversion of DMADP to IDP
2. Cells are pelleted at 4°C max speed in a 2 ml microfuge tube, supernatant is discarded unless needed for glucose or pH analysis. (Two ml of culture can be put into the tube)
   Samples can and should be frozen at this point in -80°C this helps break open cells
3. 1.5 mL (two 750 µl squirts) of ice-cold 2-propanol:100 mM NH_4HCO_3:acetonitrile pH 7.8 (1:1:3 v/v) is added followed by vortexing
4. The solution is sonicated briefly with the microtip sonicator to break up pellet and cells (Branson Sonifier 250 Output 1, Duty Cycle constant)
   You want to sonicate the sample as long as possible without heating up sample.
5. Samples pipetted into a 2 ml glass vial with septum top (Supelco ABC vial 27488-U). Samples can be frozen at -80°C at this point. Freezing will not break glass vial.
6. Frozen vial is transferred to the SpeedVac rotor using a vial top with the septum removed. Samples are dried for three hours at low heat
7. The samples are stable at this point and can be stored at -80°C but the rest of the steps must be carried out without significant delay between the steps
8. The residue is resuspended in 500 µL ice-cold 8.5% (v/v) H_3PO_4
9. The vial is sealed and then put in a dry bath at 70°C for 60 min
10. The reaction is quenched by putting the vials on ice and then adding 200 µL of 9.2 M NaOH, vials are kept on ice until sampled
11. 1 mL of head space gas is withdrawn by gas tight syringe with ice cold displacement water added from a second syringe and injected onto a GC or FIS
12. The results are multiplied by 2.26*/ 1 to account for taking 1 mL of the total volume of the vial.

Brüggemann N, Schnitzler JP (2002) Diurnal variation of dimethylallyl diphosphate concentrations in oak (Quercus robur) leaves. Physiologia Plantarum **115:** 190-196
Copolovici LO, Niinemets U (2005) Temperature dependencies of Henry's law constants and octanol/water partition coefficients for key plant volatile monoterpenoids. Chemosphere **61:** 1390-1400
Henneman L, van Cruchten AG, Kulik W, Waterham HR (2011) Inhibition of the isoprenoid biosynthesis pathway; detection of intermediates by UPLC-MS/MS. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids **1811:** 227-233

* This number is calculated as follows: The total gas volume is 1.3 mL while the liquid volume is 0.7 mL. The Henry's constant (1836 Pa m^3 mol^{-1} at 0°C) can be converted to a gas/liquid ratio of isoprene per volume by dividing by 101 kPa and 25 L/mol (approximate volume of a gas at lab conditions). The result is a ratio of 0.73 moles of isoprene in gas volume relative to moles in liquid volume. The correction is therefore 1.3 + 0.7/0.73 = 2.26

DMADP Assay Reagents

100 mM NH_4HCO_3 250 ml
In 250 ml dH_2O +
1.98 g NH_4HCO_3 (FW 79.06 g/mol)

8.5% (v/v) H_3PO_4 250 ml
In 225 ml of dH_2O +
25ml 86% H_3PO_4
** remember acid into water, never the reverse **

9.2 M NaOH 250 ml
In 150 ml dH_2O +
92 g NaOH (FW 40 g/mol)
Adjust final volume to 250 ml