

Echelon Biosciences Inc.

CDP-ME Synthase (IspD) Enzymatic Assay

K-2000D

Support: echelon@echelon-inc.com

Description: 96-well assay for monitoring the activity of the CDP-ME Synthase (IspD) enzyme.

Materials Provided

Catalog #	Description	Amount
E-2000D	CDP-ME Synthase (IspD) (3.75 μ M)	2.5 μ g
K-2001D	AC Buffer	2 mL
K-2003D	MEP Substrate Solution (312.5 μ M)	500 μ L
K-2004D	Dilution Buffer	2 mL
K-2005D	Pyrophosphatase	15 μ L
K-2006D	CTP Buffer	20 μ L
K-1501	Malachite Green Solution	21 mL
K-1502b	100 μ M Phosphate Standard Solution	1 mL
K-DTT1	Lyophilized DTT	50 μ mol
---	Reaction Incubation Plate (U bottom)	1 plate
---	Plate Seal	1 seal

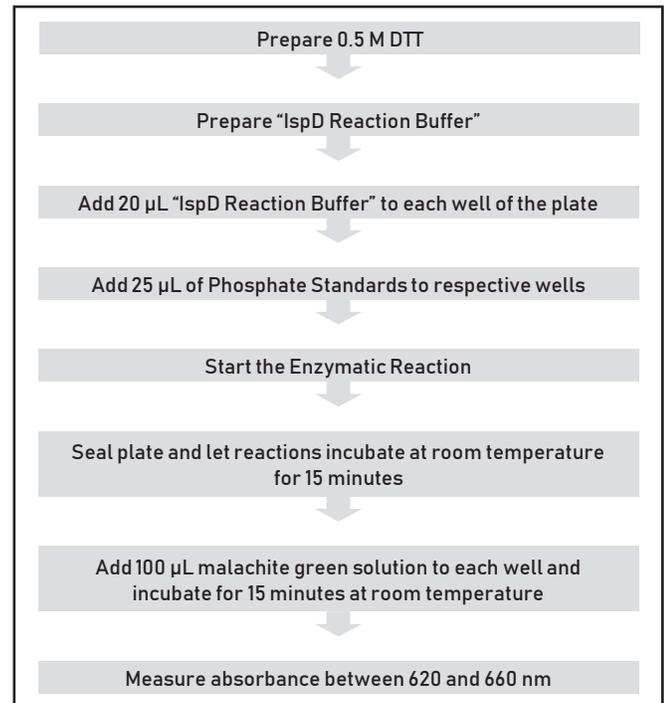
Additional Materials Provided by User:

- Pipettes (capable of delivering between 5 and 1,000 μ L)
- Multichannel pipettes
- Absorbance plate reader capable of reading at 620 nm.

Storage:

Kit can be stored unopened at -20°C for up to six months. All components and solutions should be protected from light.

Quick Protocol



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Background:

Isoprenoids comprise one of the most diverse classes of compounds found in nature. With over 50,000 different isoprenoids identified to date, they exhibit a broad range of structural complexity and are involved in a variety of biological functions [1]. Electron transport (quinones), stabilization of cell membranes (hopanoids and sterols), cell wall biosynthesis (dolichols), signal transduction (prenylated proteins), photosynthesis (chlorophylls) and modification of tRNAs are among the processes that involve isoprenoids [2]. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are the precursors for all isoprenoid compounds and two unrelated essential pathways exist in nature for their biosynthesis. These two precursors are produced by either the MVA or MEP pathway. The MVA pathway is found primarily in eukaryotes and archaea, including humans, plant cytosol and some Gram-positive bacteria, while the MEP pathway is utilized by most bacteria, including all Gram-negatives, and plant chloroplasts. Due to this natural distribution, the MEP pathway represents a promising target for development of novel antibacterial agents and herbicides [3].

In the second committed step of the MEP pathway, MEP is converted to 2-C-methyl-D-erythritol 4-phosphate (CDP-ME) by CDP-ME synthase (IspD, also referred to as YgbP) with the concomitant formation of inorganic pyrophosphate (PPi). The assay provided in this kit includes inorganic pyrophosphatase that converts the PPi generated during IspD catalysis to inorganic pyrophosphate (Pi) [4]. Pi can be detected spectrophotometrically using a malachite green solution.

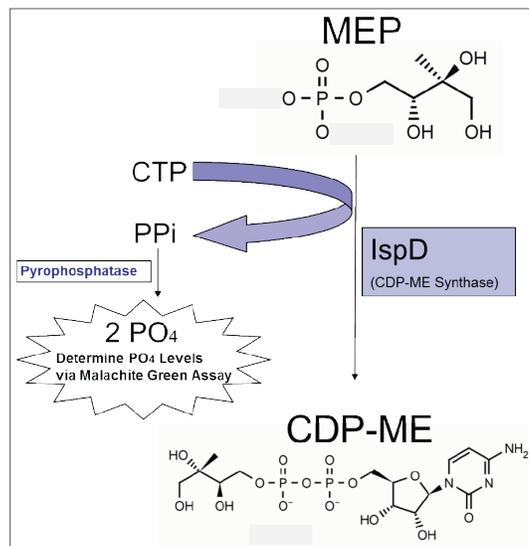


Figure 1:

Diagram of CDP-ME synthesis from MEP using CDP-ME synthase and CTP. Free inorganic pyrophosphate (Pi) can be detected using Malachite Green.

Assay Kit Notes:

1. Bring reagents to room temperature before use. Keep enzymes (E-2000D and K-2005D) on ice.
2. The amount and concentration of the IspD enzyme (E-2000D) utilized in the assay should be determined by the user.
3. The amount of IspD added to the enzymatic reaction may be adjusted in order to monitor enzyme activity.
4. Monitor enzyme progress by adding Malachite Green Solution (K-1501) to separate reactions at various times.
5. Phosphate buffers such as PBS are incompatible in this assay.
6. Carefully add malachite green solution to avoid introducing bubbles as they interfere with absorbance measurements.
7. Detergents used to clean labware may contain high levels of phosphate. Use caution either by rinsing labware with ddH₂O or by using new plasticware.
8. DMSO concentrations of up to 10% can be used without any effect on assay performance.
9. The IspD assay's linear portion of the standard curve occurs at Pi levels < 60 μM.

Assay Procedure:

Please read this entire section and the assay notes section before beginning the assay.

Preparation of Phosphate Standard Curve

Prepare the phosphate standards by diluting the 100 μM Phosphate Standard Solution (K-1502) with the Dilution Buffer (K-2004D) provided in this kit. Diluted standards should range between 0 and 60 μM per well in a volume of 25 μL (or in a similar volume as the enzyme reaction). Refer to Table 1.

Procedure - use in conjunction with Table 2

1. Prepare 0.5 M DTT by adding 100 μL ddH₂O to vial (K-DTT1). Mix thoroughly.
2. Prepare "IspD Reaction Buffer" by adding 10 μL of CDP-ME Synthase (E-2000D) solution, 10 μL Pyrophosphatase (K-2005D), 12.5 μL CTP Buffer (K-2006D), and 5 μL 0.5 M DTT (K-DTT1) to 2 mL of the AC Buffer (K-2001D). Mix thoroughly.
3. Add 20 μL "IspD Reaction Buffer" (Step 2) to each well. (Do not add to Phosphate Standard wells.)

Table 1. Phosphate Standards

Phosphate Concentration (μM)	Volume of 100 μM phosphate stock solution (μL)	Volume of Dilution Buffer K-2004D (μL)
60	60	40
50	50	50
40	40	60
30	30	70
20	20	80
10	10	90
0	0	100
Total vol	210 μL	490 μL

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References (Background):

1. Bochar, D.A.; Freisen, J.A.; Stauffacher, C.V. and Rodwell, V.W.(1999) in Comprehensive Natural Products Chemistry, (Cane, D. Ed.) Pergamon Press, Oxford, pp. 15-44.
2. Sacchettini, J.C. and Poulter, C.C. (1997) Science, 277(5333), 1788-9.
3. Testa, C.A.; Brown, M.J. (2003) Current Pharmaceutical Biotechnology, 4, 248-259.
4. Bernal, C.; Palacin, C.; Boronat, A. and Imperial, S.(2005) Analytical Biochemistry 337, 55-61.

Related Products

Products	Catalog Number
Assays and Reagents	
MEP Assay	K-2000C
CDP-MEP Synthesis Kit	K-2000E
Isoprenoid Diphosphate Reagents	
	I-0050
	I-0051
Malachite Green Assay	K-1500
MEP Pathway Intermediates	
1-Deoxy-D-xylulose 5-phosphate (DXP)	I-M050
1-Deoxy-D-xylulose (DX)	I-M050A
2-C-Methyl-D-erythritol 4-phosphate (MEP)	I-M051
2-C-Methyl-erythritol (ME)	I-M051A
4-Diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME)	I-M052
MEP Pathway Inhibitors	
FR900098	B-4202
5-Ketoclomazone	B-4101
IspF Inhibitor 1	B-4102

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