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Technical Data Sheet

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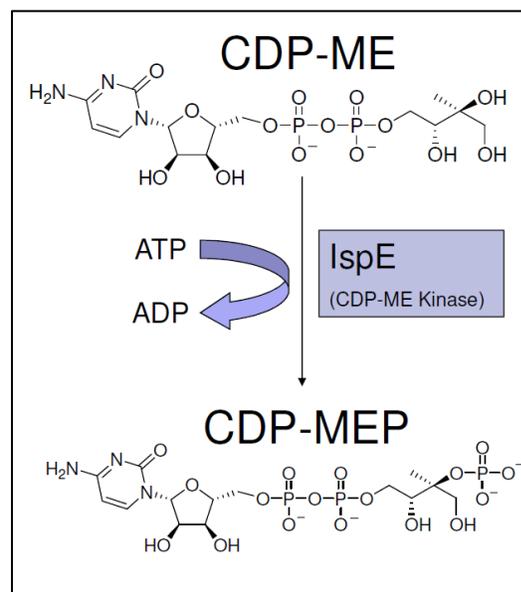
CDP-ME Kinase (IspE) (4-diphosphocytidyl-2-C-methyl-D-erythritol kinase)

Catalog #: E-2000E	Size: 20 µg	80 µg
Description:	Recombinant C-terminal His-tagged fusion protein purified from <i>E. coli</i> using Ni-NTA column chromatography.	
Size:	32.5 kDa	
Purity:	> 90% by SDS-PAGE	
Specificity:	CDP-ME kinase converts CDP-ME to CDP-MEP in the fourth step of the MEP pathway.	
Activity:	CDP-ME Kinase activity is confirmed by monitoring the consumption of ATP using CDP-ME (Cat.# I-M052) as a substrate in the CDP-MEP Enzymatic Synthesis Kit (Cat.# K-2000E).	
Formulation:	Recombinant protein is in storage buffer (50 mM HEPES, pH 7.6, 300 mM NaCl, 10 mM βME and 50% glycerol v/v).	
Storage:	Store product frozen below -20°C. Enzyme will be stable for at least 6 months at -20°C as undiluted stock. Freeze into working aliquots to avoid repeated thawing and freezing.	
QA/Product Testing:	Enzyme activity is tested and validated through the CDP-MEP Enzymatic Synthesis Kit. Enzyme purity is determined by SDS-PAGE. * (* See Certificate of Analysis)	

Background:

In the fourth step of the methylerythritol phosphate (MEP) pathway, 4-diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) is converted to 4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate (CDP-MEP) by CDP-ME kinase (IspE) in an ATP dependent reaction. The formation of CDP-MEP occurs at a 1:1 ratio with the consumption of ATP. By measuring this decrease in ATP levels the user can infer the amount of CDP-MEP generated and monitor the activity of IspE.

The MEP pathway is used by most bacteria, including all Gram-negative bacteria, for isoprenoid biosynthesis. 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 mevalonate (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].



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References:

- [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.

MEP Related Products:

	Product	General Description	Detection Mode	Cat. No.
Purified Enzymes	DXP Synthase MEP Synthase IspD Enzyme IspE Enzyme IspF Enzyme	Recombinant His-tagged fusion proteins. Purified from <i>E.coli</i> using Ni-NTA column chromatography.		E-2000DXS* E-2000DXR* E-2000D E-2000E E-2000F
MEP Pathway Substrates and Related Substrates	MEP Pathway Intermediates	1-Deoxy-D-xylose 5-phosphate (DXP) 1-Deoxy-D-xylulose (DX) 2-C-Methyl-erythritol 4-phosphate (MEP) 2-C-Methyl-erythritol (ME) 4-Diphosphocytidyl-2-C-methyl-D-erythritol (CDP-ME) 2-C-Methyl-D-erythritol 2,4-cyclophosphate (cMEPP) 1-Hydroxy-2-methyl-2-buten-4-yl 4-diphosphate (HDMAPP)		I-M050 I-M050A I-M051 I-M051A I-M052 I-M054 I-M055
	CDP-MEP Synthesis Kit	4-diphosphocytidyl-2-C-methyl-D-erythritol-2-phosphate (CDP-MEP)	Luminescence	K-2000E
	Isoprenoid Diphosphate Reagents	Isopentenyl Diphosphate (IPP) Dimethylallyl Diphosphate (DMAPP) (see our website for additional reagents)		I-0050 I-0051
MEP Pathway Activity Assays	IspD Assay	Detects CDP-ME synthase activity through detection of free phosphate (side product of coupled synthase assay)	Absorbance 620 nm	K-2000D
Antibacterial Screening Service		Whole-Cell MEP Pathway-Selective Antibacterial Screening Assay Service: MEP Pathway Inhibitor Identification Assay	Absorbance 600 nm	T-2000
Phosphatase Activity Assays	Malachite Green	Detection of free phosphate	Absorbance 620 nm	K-1500
MEP Pathway Inhibitors	Fosmidomycin	Fosmidomycin is a naturally occurring antibiotic that inhibits 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR)		B-4201
	FR-900098	FR900098, an analog of the naturally occurring antibiotic Fosmidomycin, inhibits 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR)		B-4202
	5-Ketoclofazone	5-Ketoclofazone inhibits 1-deoxy-D-xylulose-5-phosphate (DXP) synthase		B-4101
	IspF Inhibitor 1	IspF Inhibitor 1 inhibits 2-C-methyl-D-erythritol 2,4 cyclodiphosphate (cMEPP) synthase		B-4102

* Coming Soon / Check Echelon's website (www.echelon-inc.com) for additional MEP activity assays, purified enzymes, and substrates.