

Recombinant Diacylglycerol Kinase (*E. coli*)

CATALOG NUMBER: MET-5035 (1 vial)
MET-5035-5 (5 vials)

STORAGE: -80 °C; avoid freeze/thaw

QUANTITY AND CONCENTRATION: 100 µg/vial at 1.0 mg/mL in 1X PBS

SHELF LIFE: 1 year from date of receipt under proper storage conditions

Background

Diacylglycerol kinases (DGK or DAGK) is a group of enzymes that catalyze the conversion of diacylglycerol to phosphatidic acid, both key cellular lipid signaling molecules. In *E. coli*, DAGK is a small, integral membrane protein (13-15 kDa) which is structurally distinct from other kinases. DAG kinase has been widely used to quantify diacylglycerol in crude lipid extracts, but will also phosphorylate ceramide and monoglycerols.

Purity and Specific Activity

Purified recombinant DAG Kinase expressed in *E. coli* with greater than 90% purity by SDS-PAGE (Figure 1). Cell Biolabs' rDAGK has a specific activity of 1 unit/µg as determined by Cell Biolabs' DAG Kinase Activity Assay Kit (MET-5036) (Figure 2). Unit definition: One unit will phosphorylate 1.0 nmol of 1-2-dioleoyl-*sn*-glycerol per minute at pH 7.5 at 37 °C.

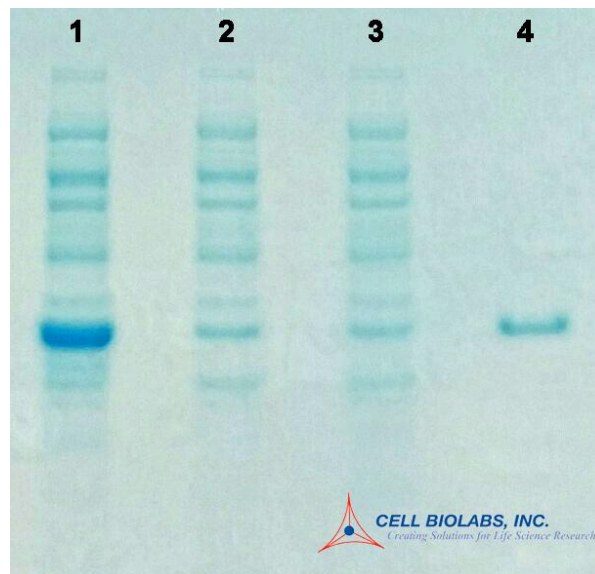


Figure 1 – Recombinant DAGK Purification. Lane 1: Whole *E. coli* lysate; Lanes 2 & 3: Washes; Lane 4: Elution.

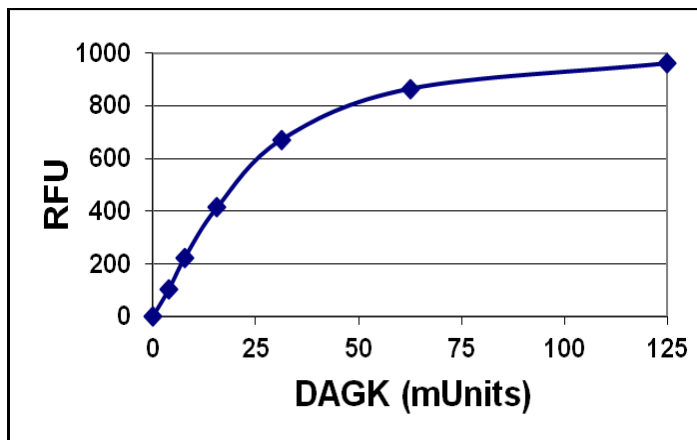


Figure 2. rDAGK Activity. The following figure demonstrates enzymatic activity of Cell Biolabs' rDAGK as tested in the DAG Kinase Activity Assay Kit (MET-5036). Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 544/590 nm filter set and 590 nm cutoff.

References

1. Topham, M.K. and Prescott, S.M. (1999) *J. Biol. Chem.* **274**:11447-11450.
2. Van Bitterswijk, W.J. and Houssa, B. (2000) *Cell Signal.* **12**:595-605.
3. Smith, R.L. et al. (1994) *J. Bacteriol.* **176**:5459-5465.

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