PRODUCT DATA SHEET

IDH1-Y139D (His)

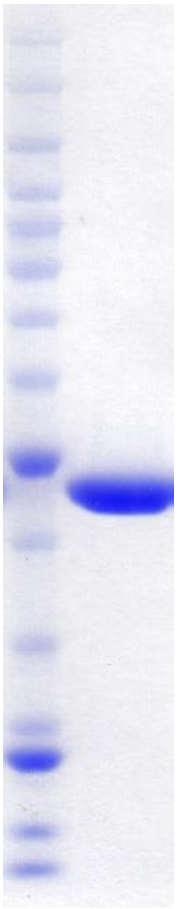
CATALOG NO.: IDH-11-328 **LOT NO.:**

DESCRIPTION: Mutant human recombinant IDH1 with aspartic acid (D) substituted for tyrosine (Y139) (otherwise contains wild-type residues 2-414; Genbank Accession # NM_005896.3; MW = 47.7 kDa) expressed with a C-terminal His-tag in *E. coli*.

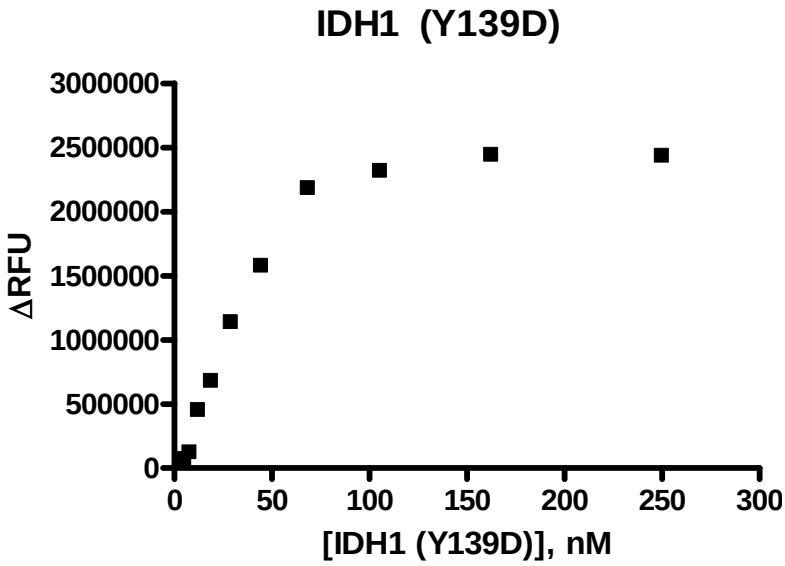
PURITY: >95% by SDS-PAGE

SUPPLIED AS: ___ µg/µL in 50 mM Tris HCl, pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted protein should be snap frozen, for example in a dry ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme is not recommended.



Coomassie blue-stained SDS-PAGE (4-12% acrylamide) of 4µg of RBC IDH1-Y139D (His). MW markers (left) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



IDH1 Y139D Activity Assay. NADPH-dependent reduction of α-ketoglutarate was determined by quantification of remaining NADPH using diaphorase/resazurin detection. The 30 µL reaction contained 15 µM NADPH, 10 mM α-KG and a variable amount of IDH1-G97D. After incubation at room temperature for 60 minutes, the reaction was quenched by the addition of diaphorase and resazurin (15 µg/ml and 30 µM respectively). The resulting fluorescence (ex. 535nm/em. 590nm) was measured using an Envison Reader (Perkin Elmer). An increase in ΔRFU represents oxidation of NADPH.

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