PRODUCT DATASHEET

IDH1-R132C (His-tagged) (Isocitrate Dehydrogenase, [NADP] cytoplasmic; Cys-132 mutant)

CATALOG NO.: IDH-11-324 **LOT NO.:**

DESCRIPTION: Mutant human recombinant IDH1 with cysteine (C) substituted for arginine (R132) (otherwise contains wild-type residues 1-414; Genbank Accession # NM_005896.3; MW = 47.7 kDa) expressed with a C-terminal His-tag in *E. coli*. IDH1-R132C is a cancer-associated, gain-of-function mutant form of IDH1 which can catalyze the reduction of α-ketoglutarate, at the expense of NADPH oxidation, to form the 'oncometabolite' and inhibitor of various α-ketoglutarate-dependent enzymes, 2-hydroxyglutarate (2-HG)[1].

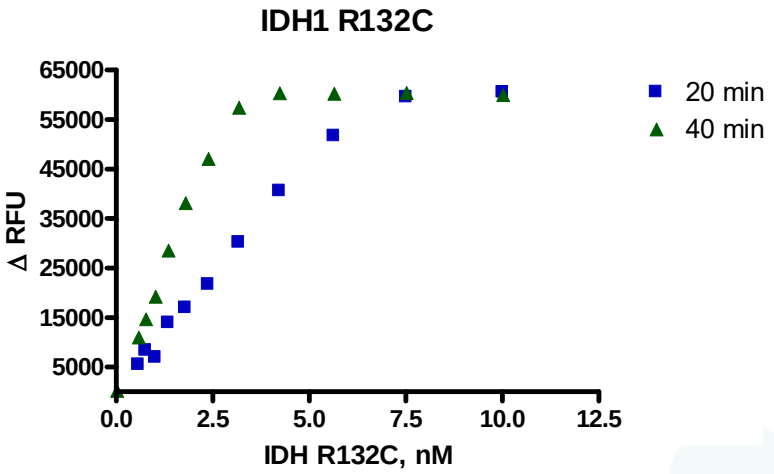
PURITY: >95% by SDS-PAGE

SUPPLIED AS: _ µg/µL in 50 mM HEPES, pH 7.5, 200 mM NaCl, 3 mM DTT, 10% glycerol

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.

REFERENCE: 1) L. Dang *et al. Nature* 2009 **462** 739;

Coomassie blue-stained SDS-PAGE (12% acrylamide) of 2µg of RBC IDH1-R132C (His). MW markers (right) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15, 10 kDa.



IDH1 R132C Activity Assay. NADPH-dependent reduction of α-ketoglutarate was determined by quantification of remaining NADPH using diaphorase/resazurin detection. The 20 µL reaction contained 15 µM NADPH, 0.5 mM α-KG and a variable amount of IDH1 R132C. After incubation at room temperature for 20 or 40 minutes, the reaction was quenched by the addition of diaphorase and resazurin (15 µg/ml and 25 µM respectively). The resulting fluorescence (ex. 528nm/em. 590nm) was measured using a Synergy H4 plate reader (Biotek). An increase in ΔRFU represents oxidation of NADPH, where the maximum signal (~65,000 ΔRFU) represents complete oxidation of 15 µM NADPH.

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