

IDH1-R132H (His-tagged) (Isocitrate Dehydrogenase, [NADP] cytoplasmic; His-132 mutant)

CATALOG NO.: IDH-11-323

LOT NO.:

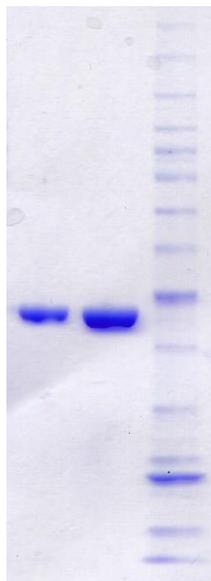
DESCRIPTION: Mutant human recombinant IDH1 with histidine (H) substituted for arginine (R132) (otherwise contains wild-type residues 1-414; Genbank Accession # NM_005896.3; MW = 47.7 kDa) expressed with a C-terminal His-tag in *E. coli*. IDH1-R132H is a cancer-associated, gain-of-function mutant form 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

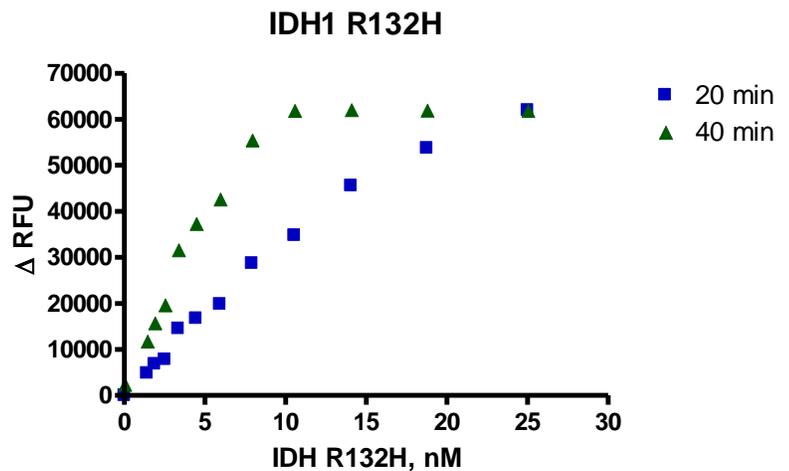
SUPPLIED AS: $_ \mu\text{g}/\mu\text{L}$ in 50 mM HEPES, pH 7.5, 200 mM NaCl, 3 mM DTT, 10% glycerol

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 (4-12% acrylamide) of 1 μg and 2 μg of RBC IDH1-R132H (His). MW markers (right) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa.



IDH1 R132H 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, 1.5 mM α -KG and a variable amount of IDH1-R132H. After incubation at room temperature for 20 or 40 minutes, the reaction was quenched by the addition of diaphorase and resazurin (15 $\mu\text{g}/\text{ml}$ 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) corresponds to complete oxidation of 15 μM NADPH.

This product is NOT intended for therapeutic or diagnostic use in animals or in humans.