UBE2E3 (UBCH9), His-Tag Recombinant

Catalog: 79371
Lot: 240111

Product Information

Description:	Recombinant human UBE2E3 (ubiquitin-conjugating enzyme E2 E3), full length. This construct contains an N-terminal His-tag. This protein was affinity purified.
Species:	Human
Construct:	UBE2E3 (His-Full Length)
Concentration:	0.10 mg/ml
Expression System:	*E. coli*
Purity:	≥90%
Format:	Aqueous buffer solution.
Formulated In:	50 mM Sodium Phosphate, pH 7.0, 300 mM NaCl, 150 mM Imidazole, 0.1 mM PMSF, 0.25 mM DTT, and 25% glycerol
MW:	25 kDa
Genbank Accession:	NM_006357
Stability:	At least 6 months at -80°C.
Storage:	-80°C
Instructions for Use:	Thaw on ice and gently mix prior to use. DO NOT VORTEX. Perform a quick spin before opening. Aliquot into small volumes and flash freeze for long term storage. Avoid multiple freeze/thaw cycles.
Specific Activity:	17 pmol/min/µg
Assay Conditions:	UBE2E3 activity was measured by using wild-type ubiquitin diluted in Ubiquitination Buffer to a working concentration of 170 ng/µl, in an AMP-Glo™ based assay (Promega #V5011). The reaction was initiated by mixing increasing amounts of UBE2E3 with UBA1 (ubiquitin-like modifier activating enzyme 1), BIRC3 (baculoviral IAP-repeat containing protein 3) and ATP in Ubiquitination Buffer (40 mM Tris (pH 7.5), 20 mM $MgCl_2$, 0.1 mg/ml BSA and 0.5 mM DTT). First a 2x Master Mix containing 170 ng/ µl of ubiquitin, 15 ng/µl UBA1, 40 ng/µl of BIRC3 and 50 µM ATP was prepared. 5 µl of 2x Master Mix were added to 5 µl of UBE2E3 serial dilutions prepared at 2x the final desired concentrations in Ubiquitination Buffer, and incubated for 60 minutes at 37°C. The plate was equilibrated to room temperature (RT). 10 µl of AMP-Glo™ Reagent I were added, and the plate was incubated at RT for another 60 minutes. 20 µl of Detection Solution were added to each reaction and the plate was incubated at RT for 30 minutes. The plate was read on a GloMax plate reader (Promega #E7031) using the KinaseGlo Luminescence Protocol. The Enzyme Specific Activity (SA) was calculated as follows: [[(Concentration of AMP generated in µM) x (Reaction volume in µl)]/ [(Reaction time in min) *(Enzyme amount in mg)]]* 10^{-3}. The blank was determined from a “no enzyme” sample by replacing UBE2E3 with an equal volume of Ubiquitination Buffer.
Applications:	Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

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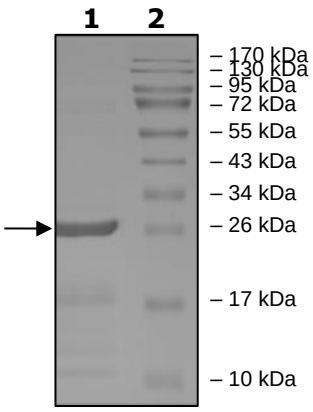
Quality Control Data

4-20% SDS-PAGE Coomassie Staining



UBE2E3 Activity