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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
H265-381G-20	20 µg
H265-381G-50	50 µg

HERC4, Active

Human recombinant protein expressed in *Sf9* cells

Catalog # H265-381G
Lot # V2408-18

Product Description

Recombinant human HERC4 (642-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The HERC4 gene accession number is NM_015601.

Gene Aliases

KIAA1593

Formulation

Recombinant proteins stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

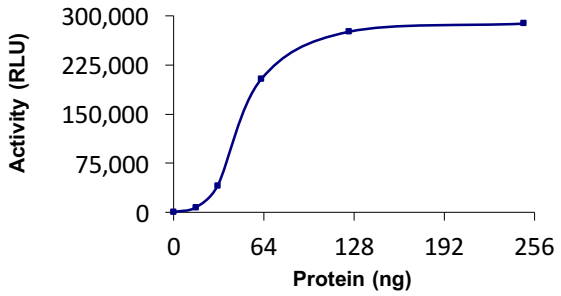
Scientific Background

HERC4 or HECT and RLD domain containing E3 ubiquitin protein ligase 4 belongs to the HERC family of ubiquitin ligases, all of which contain a HECT domain and at least 1 RCC1-like domain (RLD). The 350-amino acid HECT domain is predicted to catalyze the formation of a thioester with ubiquitin before transferring it to a substrate, and the RLD domain is predicted to act as a guanine nucleotide exchange factor for small G proteins. HERC4 is highly expressed in adult and fetal brain and in all specific brain regions examined.

References

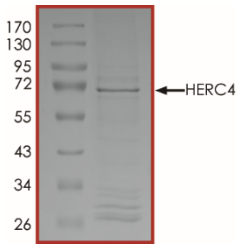
1. Hochrainer, K. et al: The human HERC family of ubiquitin ligases: novel members, genomic organization, expression profiling, and evolutionary aspects. Genomics 85: 153-164, 2005
2. Nagase, T. et al: Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. DNA Res. 7: 273-281, 2000

Specific Activity



The specific activity of HERC4 was determined to be **6 nmol /min/mg** as per activity assay protocol.

Purity



The purity of HERC4 was determined to be **>80%** by densitometry, approx. MW **71 kDa**.

HERC4, Active

Recombinant human protein expressed in *Sf9* cells

Catalog #	H265-381G
Specific Activity	6 nmol/min/mg
Lot #	V2408-18
Purity	>80%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes

Active HERC4 (Catalog #: H265-381G), UBA1 (Catalog #: U201-380G) and UBE2D1 (Catalog #: U213-380H) diluted with Ubiquitination Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active HERC4 for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM $MgCl_2$, 0.1mg/ml BSA. Add 0.5mM DTT prior to use.

AMP-Glo™ Assay (Promega, Catalog #: V5011)

AMP, 10 mM
Ultra Pure ATP, 10mM
AMP-Glo™ Reagent I
AMP-Glo™ Reagent II
Kinase-Glo™ One Solution

Substrate (Catalog #: U06-54N)

Wild-type ubiquitin protein diluted with Ubiquitination Buffer to a working stock of 170ng/µl (20µM).

Assay Protocol

The HERC4 assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin conjugation is proportional to the generated AMP, and the presence of all components of the Ub conjugation machinery (Ub, E1, E2, and E3) is required for maximal activity of the system.

Step 1. Thaw the active HERC4, UBA1, UBE2D1 and ubiquitin on ice, and all AMP-Glo™ components except AMP-Glo™ Reagent II at room temperature. Keep AMP-Glo™ Reagent II on ice.

Step 2. Prepare the following working solutions with Ubiquitination Buffer:

- 2X Reaction Cocktail: 170ng/µl ubiquitin + 15ng/µl UBA1 + 14ng/µl UBE2D1 + 50µM ATP
- 2X final concentration of Active HERC4

Step 3. In a half-area white 96-well plate, add the following components to bring the initial reaction volume to 10 µl:

Component 1. 5 µl of 2X Reaction Cocktail

Component 2. 5 µl of 2X Active HERC4

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of Ubiquitination Buffer.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Seal the plate with a plate seal and incubate at 37°C for 60 minutes

Step 5. Equilibrate plate to room temperature. Add 10 µl of AMP-Glo™ Reagent I to all wells, mix by shaking for 1-2 minutes. Incubate the plate at room temperature for 60 minutes.

Step 6. Prepare AMP Detection Solution by adding AMP-Glo™ Reagent II to Kinase-Glo™ One Solution at a 1:100 volume ratio. Add 20 µl of the Detection Solution to all wells. Mix for 1-2 minutes and incubate at room temperature for 30 minutes

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 8. Using the AMP standard curve, determine the concentration of AMP produced (µM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine AMP amount from RLUs, see AMP-Glo™ Assay protocol at Promega's website: www.promega.com/protocols

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AMP](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$

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