

Produktinformation



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Cas13a, His-Tag (L. wadei) Recombinant

Catalog: 101630 Lot: 230110

Product Information

Description: Recombinant L. wadei LwCas13a (type VI-A CRISPR-associated RNA-guided

ribonuclease Cas13a), encompassing amino acids 1-1152. This construct contains a C-terminal His Tag. Cas13a is an RNA-guided endonuclease that belongs to the class 2 type VI CRISPR-Cas system. When activated, Cas13a induces collateral cleavage of

nearby non-targeted RNAs in a nonspecific manner.

Species: Leptotrichia wadei

Construct: Cas13a (1-1152-His) (L. wadei)

Concentration: 0.30 mg/ml Expression System: E. coli
Purity: ≥90%

Format: Aqueous buffer solution.

Formulated In: 50 mM Tris, pH 7.5, 600 mM NaCl, 2 mM DTT, and 10% glycerol

MW: 125 kDa

Genbank Accession: WP_021746774

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.

Assay Conditions: Varying amounts of LwCas13a activity was measured using a CRISPR-based fluorescent

reporter assay for optimal results. Target RNA cutting and collateral RNase activity was activated using RNA-guided RNA Binding to Cas13a. Emission of fluorescent signal is

due to the degradation of the reporter substrate upon cleavage.

Active Cas13 was thawed on ice while 1X Reaction Buffer containing 20 mM HEPES, pH 7.0, 50 mM KCl, 5 mM MgCl₂, and 0.1 mg/ml BSA, guide RNA (target-specific spacer sequence), target RNA activator (complementary sequence to crRNA) were equilibrated to room temperature. Next three working solutions of Active Cas13 (4X final concentration) guide RNA (4X final concentration) and activator/reporter mix containing RNA activator and reporter substrate (2X final concentration), were prepared using 1X Reaction Buffer. 10 μl of 4X active Cas12 and 10 μl of 4x guide RNA were then preincubated in half the area of a solid black 96-well plate for 5 minutes at room temperature. After preincubation, 20 μl of 2X activator/reporter mix was added to plate and placed on shaking incubator for 1 min. The plate was then sealed and incubated at 37°C for 10-30 minutes. Plate was then equilibrated to room temperature, plate sealer removed and fluorescence read on a microplate reader. Negative control was measured by replacing enzyme working solution with equal volume of assay buffer.

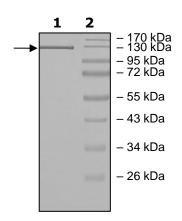
Applications: Useful for the study of enzyme kinetics, screening inhibitors, and selectivity profiling.



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Quality Control Data

4-20% SDS-PAGE Coomassie Staining



LwCas13a Collateral Cleavage Activity

