Cas13a (L. buccalis) Recombinant

Catalog: 101629
Lot: 240130

Product Information

Description:	Recombinant L. buccalis LbuCas13a (type VI-A CRISPR-associated RNA-guided ribonuclease Cas13a), tag free. 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 buccalis
Construct:	Cas13a (Full Length) (L. buccalis)
Concentration:	0.20 mg/ml
Expression System:	*E. coli*
Purity:	≥90%
Format:	Aqueous buffer solution.
Formulated In:	50 mM Tris-HCl, pH 7.5, 600 mM NaCl, 2 mM DTT, and 10% glycerol
MW:	120 kDa
Uniprot Number:	C7NBY4
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 LbuCas13a 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 $MgCl_2$, 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 DNA cleavage, targeted genome editing, the study of enzyme kinetics, screening inhibitors, and selectivity profiling.

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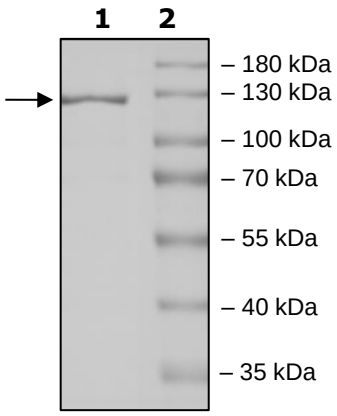
Quality Control Data

4-20% SDS-PAGE Coomassie Staining



LbuCas13a Nuclease Activity