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Data Sheet

Purified AapCas12b Protein

SKU No.: 40-1212, 40-1220, 40-1225

Description	AapCas12b is a type V CRISPR-associated protein that complexes with guide RNA (gRNA) to form a stable ribonucleoprotein (RNP) that enables efficient and precise genome editing in mammalian cells.
Purity	>90% (SDS-PAGE analysis)
Enzyme Source	*Alicyclobacillus acidiphilus* Cas12b is expressed in *E. coli* with two C-terminal nuclear localization signals (NLS) and is purified with our CLīM technology.
Storage Buffer	10 mM Tris-Cl pH 8.0, 250 mM NaCl, 50% glycerol
MW	134.5 kDa
Storage/Shipping Concentration	~ 5 mg/mL
Shipping Conditions	Dry Ice
Recommended Storage Conditions	-80°C
Endotoxin	Not Tested

Product Data

Figure 1.

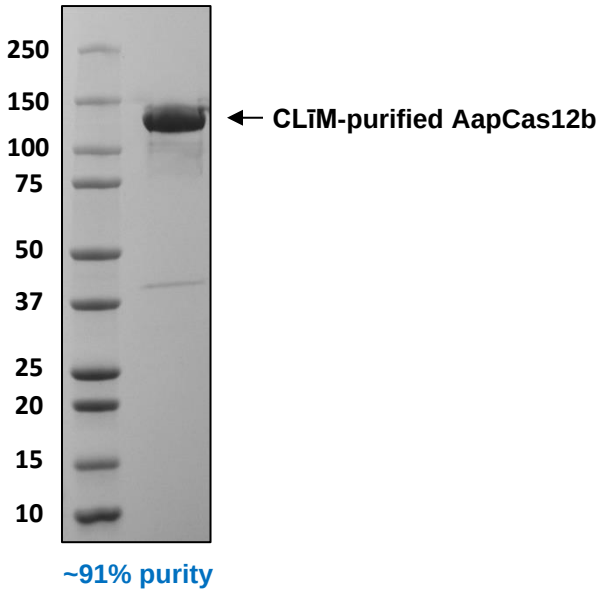

Figure 1. Purification of AapCas12b with CLīM system results in ≥90% purity.

Figure 2.

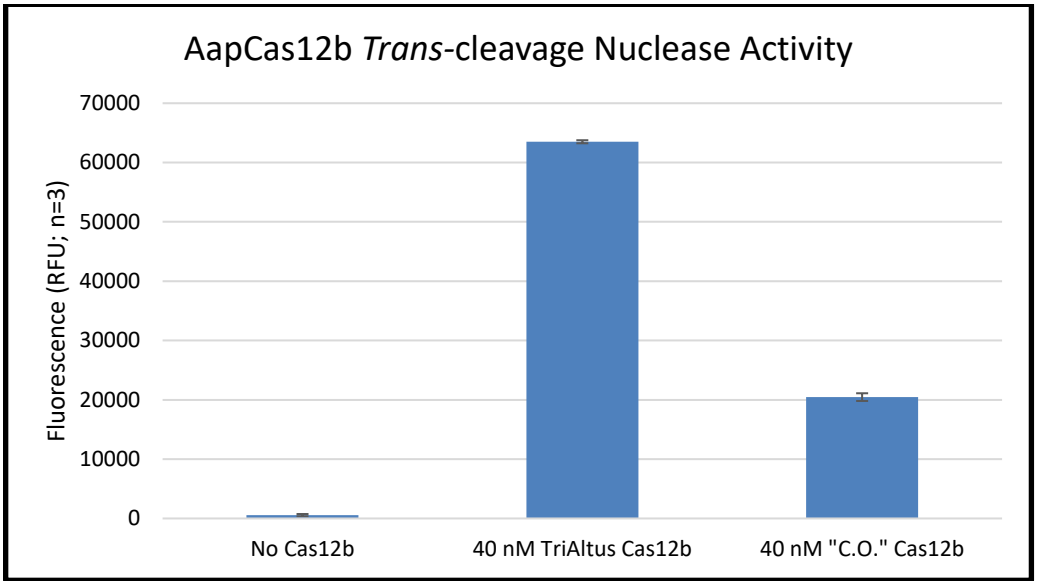

Figure 2. *CLīM-purified AapCas12b's trans-cleavage nuclease activity compared to commercially obtained ("C.O.") AapCas12b.* Upon hybridization of the Cas12b/sgRNA RNP to the target, dsDNA activator, Cas12b becomes active to cleave the activator as well as to *trans*-cleave nearby nonspecific ssDNA. Activated Cas12b cleaves dual-labeled fluorophore and quencher (FQ)-ssDNA Reporter, which releases the fluorophore to emit a fluorescent signal. Raw RFU values from each reaction were corrected by subtracting the background fluorescence value of the appropriate "no activator" control. The average of the corrected RFU values and SEM are reported. Each condition was performed in technical triplicates.

AapCas12b obtained from a commercial source or *CLīM*-purified AapCas12b was combined with annealed sgRNA for 10 minutes at room temperature to create functional AapCas12b RNP complexes. Reaction buffer was used in the place of protein for the "No Cas12b" control. A mixture of target dsDNA activator and a nonspecific FQ-ssDNA reporter was added to the designated RNP reactions. The reaction proceeded for 30 minutes at 37°C, equilibrated to room temperature for 10 minutes, and the fluorescence was measured. The reaction systems comprised the following: 0 or 40nM AapCas12b, 44nM sgRNA, 0 or 1 nM Activator, 100 nM FQ-Reporter and 1x NEBuffer r2.1 (New England Biolabs).

For research use only.
Not for diagnostic or therapeutic use.

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