

Comparison of Alt-R S.p. Cas9 nuclease with its variants

	Alt-R S.p. Cas9 Nuclease	Alt-R S.p. HiFi Cas9 Nuclease	Alt-R S.p. Cas9 D10A Nickase	Alt-R S.p. Cas9 H840A Nickase
Description	Wild-type Cas9 with high genome editing potency that is simple to use and economical	Cas9 variant with improved specificity based on reduced off-target effects, while preserving high on-target activity	Cas9 variant with a mutation in the RuvC domain that disables cleavage of the non-target strand	Cas9 variant with a mutation in the HNH domain that disables cleavage of the target strand
DNA cleavage	Both strands	Both strands	Target strand	Non-target strand
Suggested use	First choice for most CRISPR genome editing projects	Ideal for experiments that are sensitive to off-target events and require a high level of editing efficiency	May be beneficial for homology-directed repair (HDR) experiments, but requires two suitable cutting sites within an optimal distance of each other	
Molecular weight	162,200 g/mol			
Amount provided	100 µg or 500 µg			
Concentration	10 mg/mL (62 µM) in 50% glycerol			
Shipping conditions	Dry ice			
Storage conditions	-20°C at stock concentration			
Dilution	Dilute in Opti-MEM® medium (Thermo Fisher) or PBS before use			

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Comparison of CRISPR genome editing using Cas9 vs. Cas12a (Cpf1)

	Cas9 system	Cas12a system
Applications	General genome editing	<ul style="list-style-type: none"> For species with AT-rich genomes For regions with limiting design space for use of the CRISPR-Cas9 system
Ribonucleoprotein components	<ul style="list-style-type: none"> gRNA options: <ol style="list-style-type: none"> crRNA and tracrRNA sgRNA Cas9 endonuclease 	<ul style="list-style-type: none"> crRNA Cas12a endonuclease
Alt-R CRISPR enzymes	<ul style="list-style-type: none"> Wild-type HiFi Nickases (D10A and H840A) 	<ul style="list-style-type: none"> Wild-type <i>Ultra</i> (Improved performance)
Cas9 crRNA:tracrRNA (option 1)	crRNA <ul style="list-style-type: none"> Native: 42 nt Alt-R: 35–36 nt (36 nt recommended) tracrRNA <ul style="list-style-type: none"> Native: 89 nt Alt-R: 67 nt 	—
Cas9 sgRNA (option 2)	<ul style="list-style-type: none"> Alt-R: 99–100 nt (100 nt recommended) 	—
Cas12a crRNA	—	<ul style="list-style-type: none"> Native: 42–44 nt Alt-R: 40–44 nt (41 nt recommended)
CRISPR enzyme	<ul style="list-style-type: none"> Class 2, Cas type II M.W.*: 162,200 g/mol Endonuclease domains: RuvC-like and HNH 	<ul style="list-style-type: none"> Class 2, Cas type V M.W.*: 156,400 g/mol Endonuclease domain: RuvC-like only
Double-stranded DNA cleavage	<ul style="list-style-type: none"> Wild-type and HiFi: blunt-ended cut 3 bases upstream of the protospacer sequence D10A nickase with paired crRNAs: 5' overhang H840A nickase with paired crRNAs: 3' overhang PAM site often destroyed during genome editing 	<ul style="list-style-type: none"> 5' overhanging cut on the 5' side of the protospacer sequence PAM site may be preserved after genome editing
PAM sequence†	NGG	<ul style="list-style-type: none"> TTTV for Cas12a V3 TTTN for Cas12a <i>Ultra</i>
Current recommendations for Alt-R RNP delivery	<ul style="list-style-type: none"> Electroporation ± Alt-R enhancer Microinjection Lipid-mediated transfection 	<ul style="list-style-type: none"> Electroporation with Alt-R enhancer Microinjection

* Molecular weight of Alt-R nuclease

† N = any base; V = A, C, or G

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