

## Alt-R® S.p. HiFi Cas9 Nuclease 3NLS

Highly specific genome editing, even under challenging conditions

Alt-R S.p. HiFi Cas9 Nuclease 3NLS is a high-fidelity *S. pyogenes* Cas9 protein that significantly reduces off-target effects without compromising performance—perfect for routine experiments and ideal for challenging genome editing applications.

The Alt-R S.p. HiFi Cas9 enzyme easily replaces wild-type Cas9 in existing applications, with no need for protocol changes. The enzyme is compatible with other components of the Alt-R CRISPR-Cas9 System to enable precise genome editing through the same advantageous ribonucleoprotein (RNP)-based workflow.

### benefits

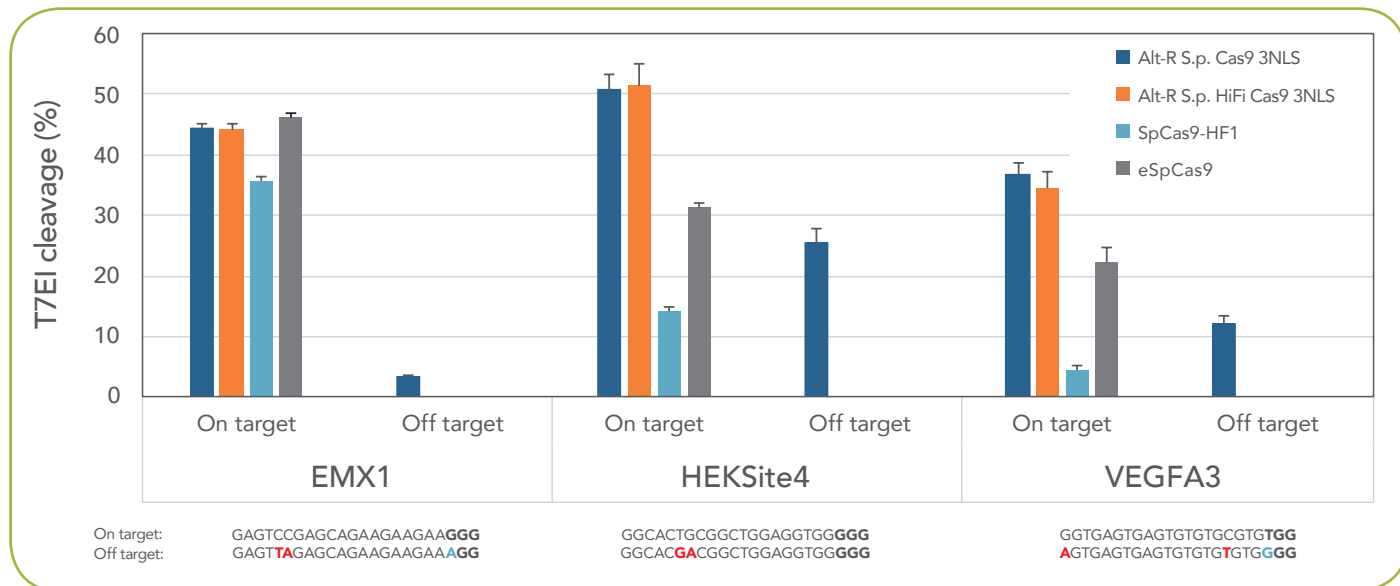
**Achieve increased specificity** with strongly reduced off-target activity

**Obtain similar high efficiency** to the market-leading Alt-R S.p. Cas9 Nuclease 3NLS

**Deliver the ribonucleoprotein efficiently** by lipofection, electroporation, or microinjection

**Avoid the toxicity and innate immune response activation** commonly observed with *in vitro* transcribed Cas9 mRNA and sgRNAs

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[www.idtdna.com/CRISPR-Cas9](http://www.idtdna.com/CRISPR-Cas9)



**Figure 1. Alt-R S.p. HiFi Cas9 Nuclease 3NLS provides consistently high on-target performance, while reducing off-target editing, when delivered as a ribonucleoprotein (RNP).** RNP complexes (1  $\mu$ M) were formed with wild-type Cas9 protein (Alt-R S.p. Cas9 Nuclease 3NLS; dark blue) and 3 high-fidelity Cas9 variants, Alt-R HiFi Cas9 (orange), SpCas9-HF1 (light blue; Kleinstiver et al., *Nature* 529:490–495), or eSpCas9 (gray; Slaymaker et al., *Science* 351:84–88), combined with an Alt-R crRNA:tracrRNA gRNA complex targeting the *EMX1*, *HEKSite4*, or *VEGFA3* loci. Complexes (10 nM) were delivered into HEK-293 cells by reverse transfection using Lipofectamine® RNAiMAX Transfection Reagent (Thermo Fisher), and DNA was extracted after 48 hr. Editing was measured by PCR amplification of the indicated on- and off-target sites, followed by cleavage with T7EI and analysis using the Fragment Analyzer™ system (Advanced Analytical). Error bars represent the standard errors of the means. The sequence of the on- and off-target sites for each crRNA are indicated at the bottom (red = mismatch in protospacer; blue = mismatch in PAM site).

## Ordering information

## CRISPR guide RNAs

Product	Size	Catalog #
Alt-R CRISPR-Cas9 crRNA	2, 10 nmol tubes or plates	Order at <a href="http://www.idtdna.com/CRISPR-Cas9">www.idtdna.com/CRISPR-Cas9</a>
	5 nmol	1072532
Alt-R CRISPR-Cas9 tracrRNA	20 nmol	1072533
	100 nmol	1072534

## HiFi Cas9 Nuclease

Product	Size	Catalog #
Alt-R S.p. HiFi Cas9 Nuclease	100 µg	1078727
	500 µg	1078728

## Control kits\*

Product	Catalog #
Alt-R CRISPR-Cas9 Control Kit, Human (2 nmol)	1072554
Alt-R CRISPR-Cas9 Control Kit, Mouse (2 nmol)	1072555
Alt-R CRISPR-Cas9 Control Kit, Rat (2 nmol)	1072556

\* Control kit components are also available individually.

## Control kit components

- Alt-R CRISPR HPRT Positive Control crRNA
- Alt-R CRISPR Negative Control crRNA #1
- Alt-R CRISPR-Cas9 tracrRNA
- Alt-R HPRT PCR Primer Mix
- Nuclease-Free Duplexing Buffer

[www.idtdna.com/CRISPR-Cas9](http://www.idtdna.com/CRISPR-Cas9)

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