

CRISPR-associated Nucleases Technical Guide



Tools to Support Discovery and Therapeutic Programs

Gene editing using CRISPR-associated nucleases, including SpCas9, offers a novel approach to treat many diseases. SpCas9 is now being evaluated in multiple preclinical programs and is rapidly advancing to clinical trials. While many vendors offer research-grade SpCas9, these nucleases are not applicable to clinical programs due to construct design and quality grade. Aldevron offers SpCas9 and AsCpf1 nucleases optimized for development and clinical applications.

In addition to wild-type SpCas9, Aldevron now offers *SpyFi*[™] Cas9 which supports gene editing with significantly reduced off-target effects. Aldevron's gene editing proteins are supported by a robust, scalable manufacturing protocol and an extensive quality package. These proteins are available in a range of quality grades from research grade to GMP, allowing for a seamless transition from research applications to the clinic.

Gene Editing Nucleases Available from Aldevron

Product	Construct Design					
SpyFi Cas9 Nuclease		sv40 NLS		SpyFi [™] Cas9		sv40 NLS
*sNLS-SpCas9-sNLS		sv40 NLS		wt SpCas9		sv40 NLS
His-sNLS-myc-SpCas9-HA-nucNLS	His	sv40 NLS	Myc	wt SpCas9	HA	nuc. NLS
sNLS-AsCpf1-sNLS		sv40 NLS		AsCpf1		sv40 NLS

**Available at research and GMP quality grades*

Quality Specifications

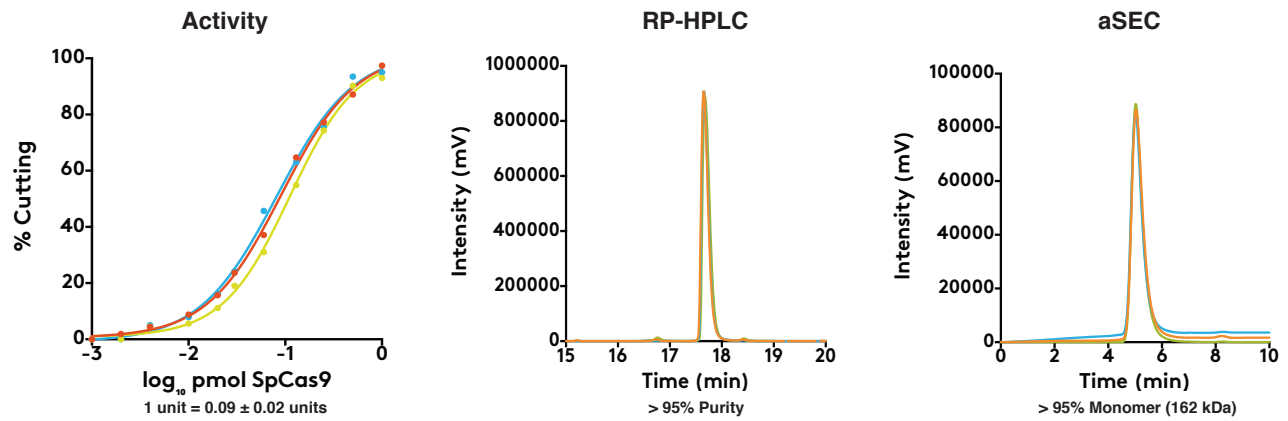
mg/mL	10.0	Endotoxin	≤ 10 EU/mg	Myco	Negative
SDS-PAGE	≥ 95%	Nuclease	≤ LOQ	Microbio	< 1 CFU/mL
RP-HPLC	≥ 95%	HC Protein	≤ 200 ng/mL	Activity	0.5 pmol cuts ≥ 50%
αSEC	≤ 5%	HC DNA	≤ 1% (w/w)		

Aldevron GMP SpCas9

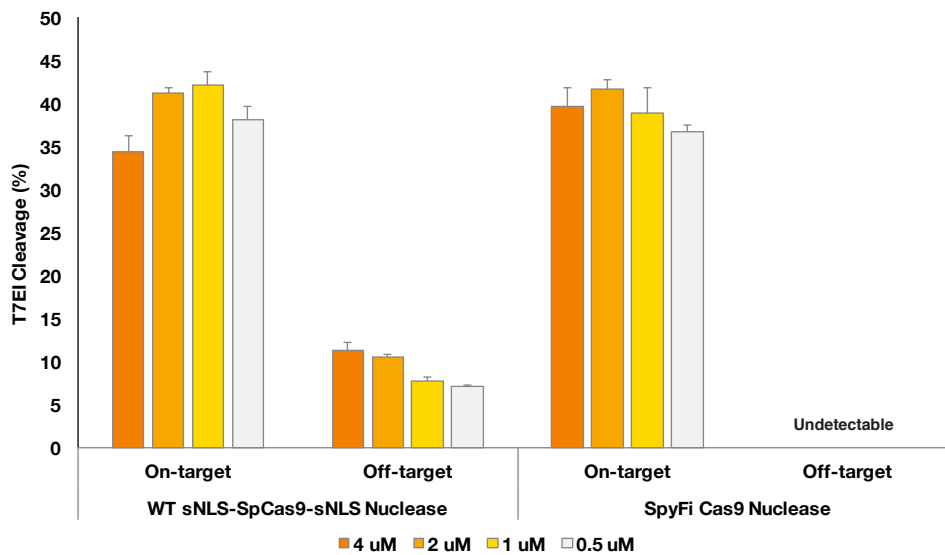
Aldevron's sNLS-SpCas9-sNLS nuclease is available as a GMP-grade catalog product (10 and 50 mg pack sizes). This product allows researchers access to SpCas9 suitable for clinical trials without having to invest in a full GMP manufacturing event.

Custom Manufacturing of CRISPR-associated Nucleases

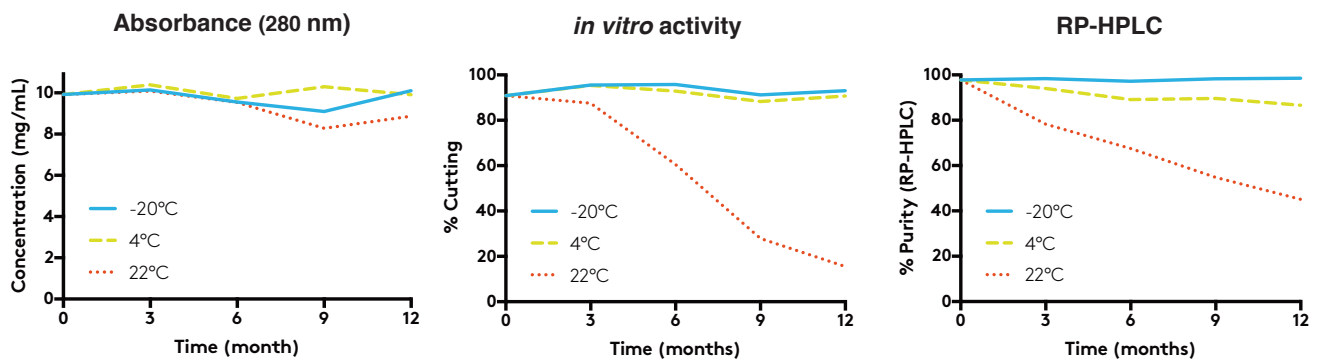
Aldevron offers custom manufacturing of CRISPR-associated nucleases, including dCas9 fusions and nickases. Manufacturing scale ranges from 10 mg to multi-gram lots. Custom nucleases are available at research, GMP-Source[™] and GMP quality grades.



▶▶ **FIGURE 1. High-quality, reproducible manufacturing.** SpCas9 lots were expressed and purified over the course of several months at different scales. Quality test results show that Aldevron’s manufacturing process consistently yields high-quality SpCas9.



▶▶ **FIGURE 2. Aldevron *SpyFi* Cas9 has reduced OTEs.** *SpyFi* Cas9 has a measurably lower frequency of off-target editing compared to wt SpCas9. Please note that Aldevron’s *SpyFi* Cas9 Nuclease is sold under license of patents and/or patents pending from Integrated DNA Technologies, Inc. (IDT). The *SpyFi* Cas9 Nuclease data shown above was generated in collaboration with IDT.



▶▶ **FIGURE 3. Aldevron sNLS-SpCas9-sNLS is stable for > 12 months in a DTT-free formulation.** sNLS SpCas9-sNLS was formulated at 10 mg/mL in 25 mM Tris, 0.3 M NaCl, 0.1 mM EDTA, 50% Glycerol, pH 7.4. Quality tests show no loss of protein, activity or purity when the nuclease is stored at -20°C.