# Cas9 mRNA

Optimized for high on-target editing and reduced off-target editing to accelerate your program timeline

- High editing efficiency
- Reduced off-target editing
- Reduced immunogenicity
- Fast shipping to your lab

## Wild type & *Spy*Fi™ high-fidelity Cas9 mRNA for discovery phase therapeutic workflows

Developed in collaboration between Integrated DNA Technologies (IDT) and Aldevron, wild type and high-fidelity *Spy*Fi™ Cas9 mRNA set a new standard for fidelity, performance, and reliability. These solutions demonstrate high editing efficiency across multiple primary and immortalized cell lines via electroporation or LNP delivery.

## Edit smarter, scale faster

### Benefits:

- Available as wild type and *Spy*Fi™ high-fidelity versions, providing high on-target and low off-target editing
- Single-vendor continuity for every phase of your therapeutic workflow (CGMP version coming soon!)
- Reduced licensing burden with enzymatically capped (eCap) mRNA
- High stability with >100 nt poly A tail and high 5′ capping efficiency
- Optimized with N1-methylpseudouridine modification for reduced immunogenicity

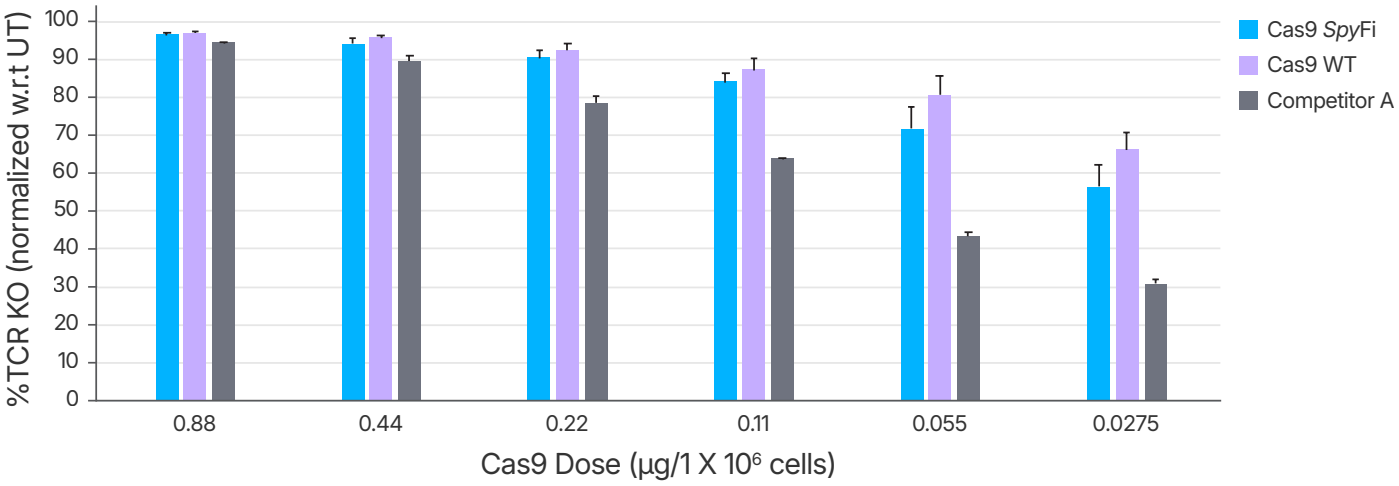
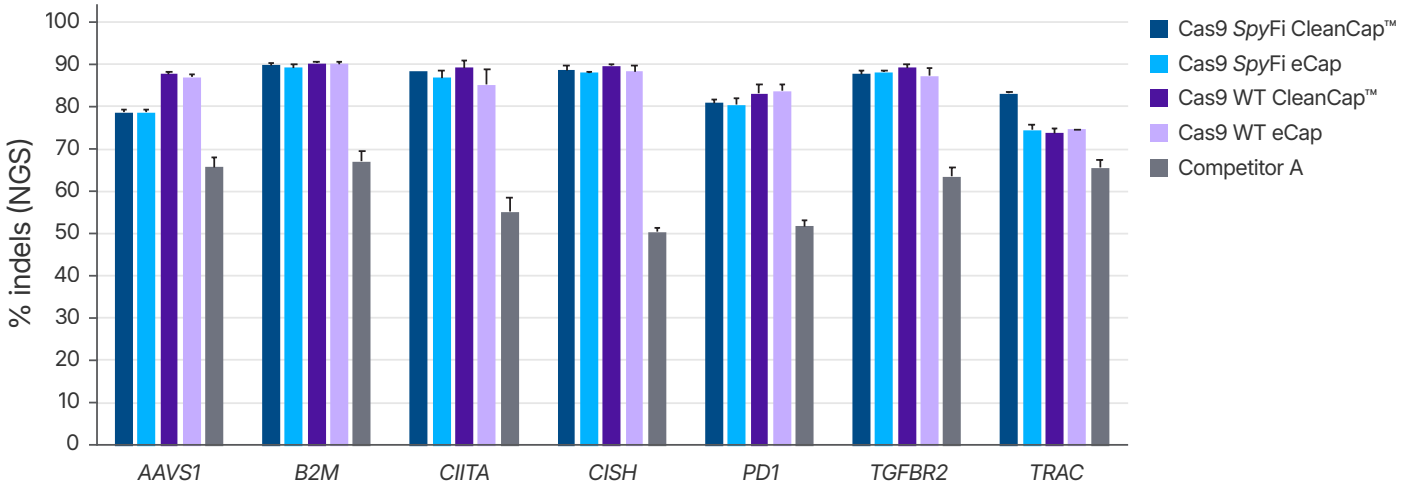



**Figure 1. T-cell receptor knockout in T-cells by LNP delivery of Cas9 mRNA and synthetic *TRAC*-targeting sgRNA.** Cas9 mRNA and synthetic *TRAC* sgRNA were encapsulated into lipid nanoparticles (LNP) using the GenVoy-ILM™ T Cell Kit on the NanoAssemblr™ Ignite instrument, following manufacturer's instructions. Briefly, RNA working solutions were prepared in the supplied formulation buffer, and Cas9 mRNA or sgRNA were individually loaded into LNPs. The lipid phase and mRNA phase were combined in the Ignite instrument and downstream processed as recommended in the instructions. Primary T-cells were thawed and activated 72 hours prior to LNP addition. Cells were seeded at 0.1 × $10^6$ cells/mL in serum-free media. Cas9 mRNA-LNPs and sgRNA-LNPS were mixed 1:1 by RNA weight and added directly to activated T-cells on Day 3. TCR knockout efficiency was assessed on Day 4 by flow cytometry after live-dead discrimination.

A. On-target editing at clinically relevant gene targets; 1 µg mRNA




B. On- and off-target editing at *HBB*

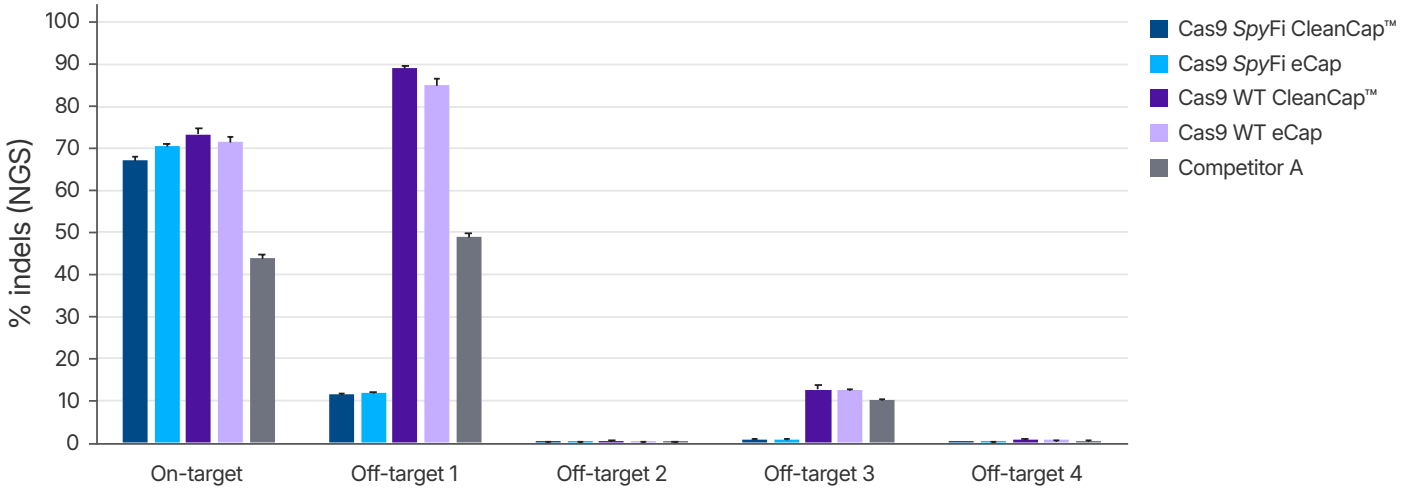



**Figure 2. On-target editing at clinically relevant research gene targets and on- and off-targeting at *HBB*.** Primary T-cells were thawed and activated 72 hours prior to nucleofection. Cas9 mRNA (1 µg) and sgRNA (2 µM) targeting *AAVS1*, *B2M*, *CIITA*, *CISH*, *PD1* and *TGFBR2* were delivered by nucleofection into T-cells using the Lonza Nucleofection™ System (**A**). Genomic DNA (gDNA) was extracted 72 hours post nucleofection and editing was assessed by NGS. Cas9 mRNA (1 µg) and sgRNA (2 µM) targeting *HBB* was delivered by nucleofection into T-cells using the Lonza Nucleofection™ System (**B**). The gDNA was extracted 72 hours post nucleofection and editing was assessed by NGS.

## Available products

Enzymatically capped mRNA encoding Cas9 from *S. pyogenes*. Contains 5′ and 3′ UTR, >100 nt polyA tail, nuclear localization sequence (NLS), and N1-methylpseudouridine modified. Provided at 1 mg/mL in 1 mM sodium citrate buffer, pH 6.4

| Product | Quantities available |
|---|---|
| *S.p.* Cas9 mRNA | 20 µg, 100 µg, & 1 mg |
| *Spy*Fi™ Cas9 mRNA | 20 µg, 100 µg, & 1 mg |

For more information, visit **idtdna.com/Cas9mRNA**