Sigma-Aldrich®
Lab & Production Materials

Data Sheet

SygRNA® Synthetic Guide Primary T-cell Editing

Genome editing experiments have never been easier or more efficient than with SygRNA® synthetic guide RNAs and Sigma-Aldrich® Cas9 proteins. However, some cells can be more difficult to edit than others. That's why we have extensively validated our reagents and protocols to work in even the most challenging of applications, including in primary T-cells (**Figure 1**), which present a number of significant challenges to overcome when attempting to knock-in or knock-out a construct or gene. With SygRNA® guides we enable editing in primary human T-cells at efficiencies and specificities that exceed two-part guide systems (**Figure 2**). In both primary T-cells and PBMCs, SygRNA® guides can be introduced as highly specific paired nickase ribonucleoprotein (RNP) complexes, or as single site Cas9 RNPs to get complete knockouts of the PD-1 gene, as measured by PD-1 expression by NGS (**Figures 3a & 3b**). SygRNA® guides can be rapidly designed for any gene as either a single target RNA or as part of the paired nickase system. **When you need to be certain, SygRNA® synthetic guides are the only sgRNAs backed by a 100% performance warranty - whether a predesigned or custom sequence, your guides are guaranteed to work!**

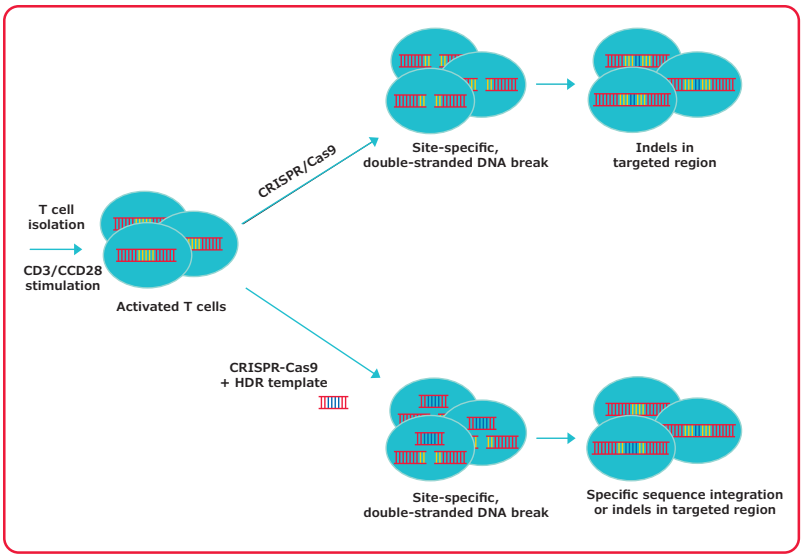

Figure 1. Application of CRISPR for the editing of primary human T-cells and PBMCs. This has proven to be an extremely powerful tool in the development of new clinical therapies. However, editing these cells can often prove difficult. The high editing efficiency produced with SygRNA® guides in difficult applications is a key benefit to researchers.

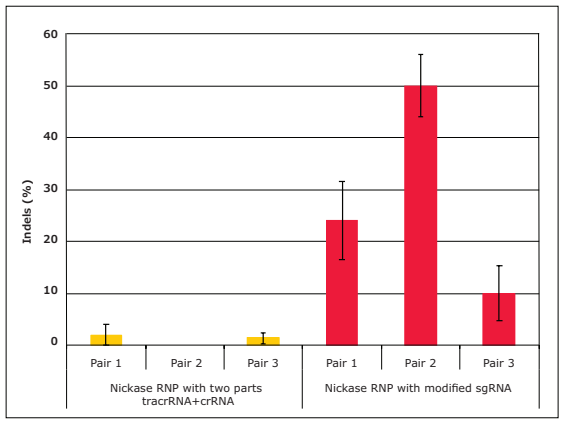

Figure 2. SygRNA® single guides significantly outperform two-part guides. Here, paired nickase RNPs containing either modified one-part sgRNA (red) or two-part tracrRNA+crRNA (yellow) were nucleofected into primary CD8+ T cells. Averages from three biological replicates are plotted with error bars representing one standard deviation.

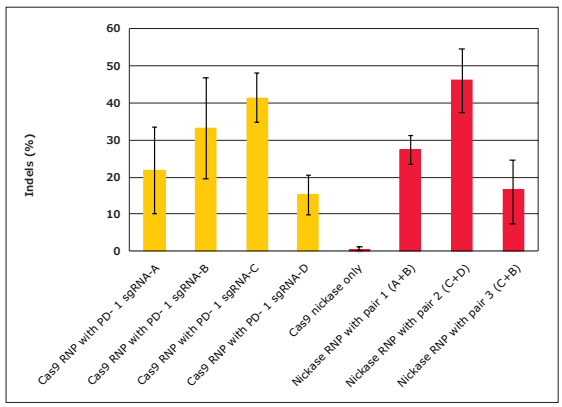

Figure 3a. Paired nickase SygRNA® guide complexes deliver superior editing efficiency. Graph showing the comparison of editing with paired nickases (red) against standard SpCas9 RNPs (yellow) in human primary T-cells and PBMCs. Indels were confirmed through NGS sequencing.

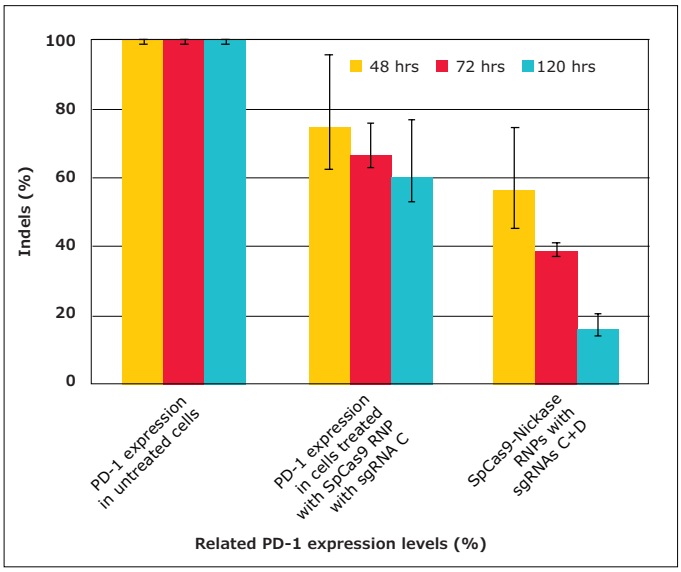

Figure 3b. Paired nickase RNPs significantly outperform SpCas9 RNPs in human primary T-cells and PBMCs. PD-1 expression levels between paired nickase RNP, along with nickase RNP containing single sgRNA or SpCas9 RNP, were delivered into human primary PBMCs. Cells stained with anti-PD-1 antibody and sorted by flow-cytometry show high efficiency KO of PD-1.

Custom Quantities and Modifications

SygRNA® sgRNAs are available in a standard quantity of 3 nmol of HPLC purified material, either unmodified or with stabilizing modifications at the 5′ and 3′ ends. Larger quantities and customized modification patterns are available upon custom request. Please contact us for additional information.

Product Guarantee

We are so confident in the performance of our SygRNA® products, that we fully guarantee the quality and performance of any guide we produce, including custom sequences. If your SygRNA® guides do not yield detectable cleavage at the intended target site, we will provide a one-time replacement, free of charge.

To qualify for this guarantee, simply send an image or sequencing data from a single experiment demonstrating detectable cleavage using one of our positive controls, side-by-side with the negative results from your SygRNA® guide. To receive your replacement, simply email oligotechserv@milliporesigma.com and include sample data from a representative experiment (T7E1, TIDE, or NGS).

Product Availability

Product Description	Format	Quantity	Purification	Modifications	Ordering
Predesigned Synthetic RNA – single guide RNA (sgRNA)	sgRNA	3 nmol, 5 nmol	HPLC	Unmodified, 3x MS*	**VC40003**
Custom Synthetic RNA – single guide RNA	sgRNA	3 nmol, custom	HPLC	Unmodified, 3x MS*	**VC40003**
Predesigned Synthetic RNA - crRNA	crRNA	2 nmol, 5 nmol	HPLC, desalted	Unmodified, 3x MS*	**VC40003**
Custom Synthetic RNA – crRNA	crRNA	2 nmol, 5 nmol, custom	HPLC, desalted	Unmodified, 3x MS*	**VC40003**
Standard TRACR RNA for S. pyogenes Cas9	tracrRNA	5 nmol	HPLC	Unmodified	**TRACRRNA05N**
Modified TRACR RNA for S. pyogenes Cas9	tracrRNA	5 nmol	HPLC	3x MS*	**TRACRRNAMOD**
Custom TRACR RNA	tracrRNA	5 nmol, custom	HPLC	Unmodified, 3x MS*	**REQUEST**
96 or 384 well-plates	crRNA, sgRNA	Custom (inquire)	HPLC, desalted	Unmodified, 3x MS*	**CUSTOM**

* Chemically modified synthetic gRNAs containing stabilizing 2′-O-methyl and phosphorothioate linkages

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