SureGuide Purified Chemically Synthesized CRISPR Guide RNA

Part Number	Product Name
G7250B	SureGuide Purified sgRNA, 100 µg, 90-120 nt
G7250C	SureGuide Purified sgRNA, 200 µg, 90-120 nt
G7250D	SureGuide Purified sgRNA, 500 µg, 90-120 nt
G7250E	SureGuide Purified sgRNA, 1 mg, 90-120 nt

Agilent SureGuide Purified Chemically Synthesized CRISPR Single Guide RNAs (sgRNAs) are produced using fully custom de novo synthesis, including the target and tracrRNA sequences. SureGuide Purified sgRNAs are manufactured under ISO 13485 standards using a proprietary chemistry and purification method for a high quality, highly reproducible, and streamlined process.

SureGuide Purified sgRNAs are available in 90 to 120 nucleotide (nt) lengths and with up to 10 chemical modifications. Modifications include: 2'-O-methyl (M); 2'-O-methyl 3'-phosphorothioate (MS); Deoxy (D); or 2'-ribo 3'-phosphorothioate (S).

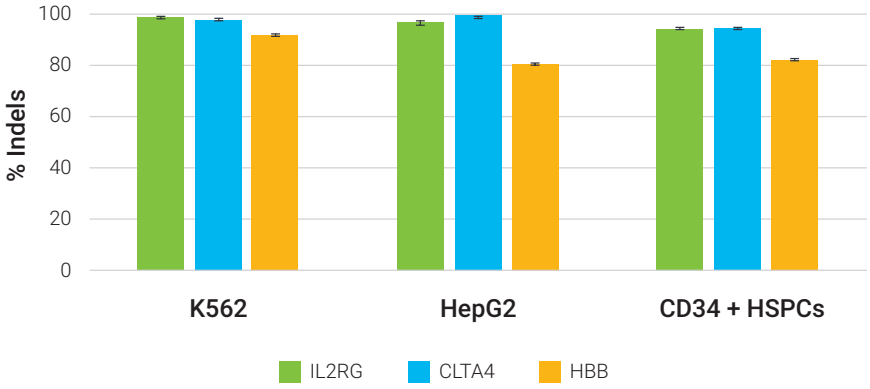

Figure 1. High levels of on-target editing are observed across multiple cell lines including K562 and HepG2 cells and in human primary cells such as C34+ HSPCs. SureGuide Purified sgRNAs containing 3xMS modifications (175 pmol) were precomplexed with *Streptococcus pyrogene* Cas9 (SpCas9) protein (50 pmol) and transfected into 0.2 million cells by electroporation. Three sgRNAs were tested in each cell line to perform editing of *IL2RG*, *CLTA4*, and *HBB* gene targets. Editing yields were measured by deep sequencing of PCR amplicons of the target locus. Bar charts display the mean of triplicate transfections with standard deviation represented as error bars.

For more information please contact:

Agilent
Trusted Answers

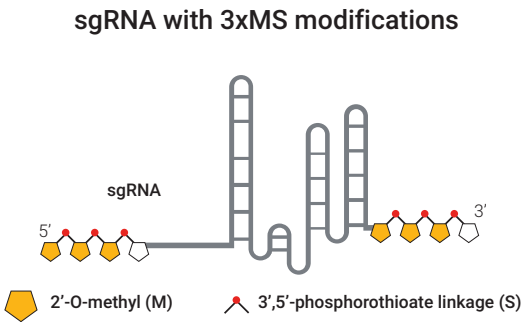

Figure 2. The Agilent patented 3xMS chemical modifications located at the 5' and 3' ends enable increased sgRNA stability and higher editing efficiency.

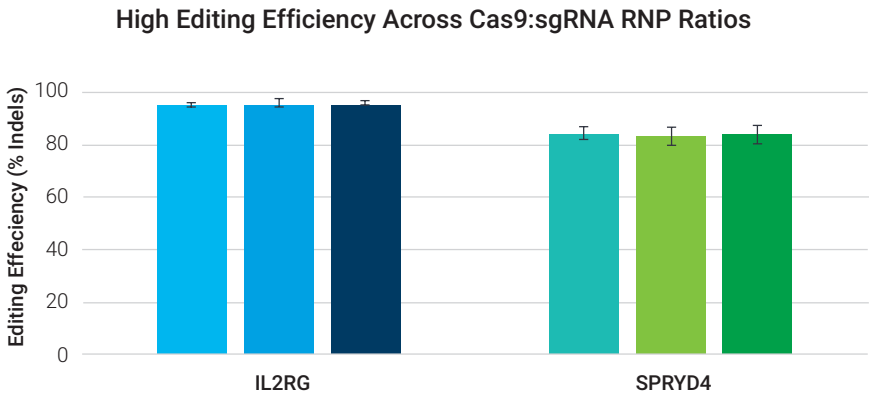

Figure 3. High levels of on-target editing are observed across multiple SureGuide designs when tested at RNP ratios ranging from more limiting to higher excess input of sgRNA. A constant amount of *SpCas9* protein (10 pmol) was precomplexed with increasing amounts of two different SureGuide Purified sgRNAs containing 3xMS modifications targeting *IL2RG* and *SPRYD4* genes at 25 pmol, 35 pmol, and 50 pmol to generate ratios of 1:2.5, 1:3.5 and 1:5, respectively. K562 cells were transfected with the different RNP mixtures using electroporation. Bar charts display the mean of quadruplicate transfections with standard deviation represented as error bars.

References

1. Dellinger, D. J,. et al. Streamlined Process for the Chemical Synthesis of RNA Using 2'-O-Thionocarbamate-Protected Nucleoside Phosphoramidites in the Solid *Phase. J. Am. Chem. Soc*. **2011**, *133* (30), 11540–11556. https://doi.org/10.1021/ja201561z.
2. Hendel, A.; et al. Chemically Modified Guide RNAs Enhance CRISPR-Cas Genome Editing in Human Primary Cells. *Nat. Biotechnol*. **2015**, *33* (9), 985–989. https://doi.org/10.1038/nbt.3290.
3. Ryan, D. E.; et al. Improving CRISPR-Cas Specificity with Chemical Modifications in Single-Guide RNAs. *Nucleic Acids Res*. **2018**, *46* (2), 792–803. https://doi.org/10.1093/nar/gkx1199.

www.agilent.com

PR7000-3392

5994-5147EN

Agilent
Trusted Answers