Synthetic sgRNA

Thank you for choosing CRISPRevolution Synthetic sgRNA for your CRISPR experiment! Our world-class guide RNA offers an unbeatable combination of quality, speed, accuracy, and price.

Step 1: Rehydrate Your sgRNA

Synthego RNA oligos ship dry at ambient temperature and remain stable for several weeks at room temperature. Please store dried RNA oligos at -20 °C for long-term storage (up to 6 months).

Be sure to work in an RNase-free environment.

Note: Please dissolve and dilute Synthego RNA based upon the labeled amount. The quantity of material present, printed on the label, is measured by UV absorbance spectroscopy at a wavelength of 260 nm, prior to dehydration. If needed, it is best practice to verify the RNA concentration using a sensitive UV absorbance spectroscopy instrument, such as a Nanodrop™. The extinction coefficient for each RNA oligo is provided in the QC document and should be used to calculate the final concentration of RNA. Small variations between the printed value and the measured value are normal.

1. Briefly centrifuge tubes or plates containing oligos to ensure RNA pellets are collected at the bottom.

2. For cell lines and primary cells: carefully rehydrate sgRNA in an appropriate nuclease-free buffer (1X TE buffer; provided) and pulse vortex for 30 seconds to ensure complete mixing.

The following table states the recommended amount of nuclease-free 1X TE buffer for six different starting quantitites of sgRNA. The final concentration of the sgRNA will be 100 µM (100 pmol/µl):

sgRNA (nmol)	1.5	3	10	20	50	100
Nuclease-free 1X TE buffer* (µl)	15	30	100	200	500	1000

* TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

For microinjection: It is critical to only hydrate and dilute sgRNA in a nuclease-free 1X Microinjection buffer (e.g., 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0; not provided).

3. Rehydrated sgRNA should be stored at -20 °C. Under these conditions, sgRNA will be stable for up to 6 months.

Step 2: Dilute Your sgRNA

1. Depending on application, sgRNA may be used directly at the rehydration concentration in 1X TE buffer, or diluted to a working stock using nuclease-free water in a sterile microcentrifuge tube or plate.

Example Protocol: Add 6 µl of 100 µM sgRNA to 14 µl of nuclease-free water to make a total volume of 20 µl of 30 µM sgRNA (30 pmol/µl).

2. Use diluted sgRNA immediately or store at -20 °C for up to 3 months (or 6 months if not repeatedly thawed).

You are now ready to use your synthetic sgRNA. We recommend forming ribonucleoprotein (RNP) complexes for your genome editing experiments to maximize editing efficiency and reduce off-target effects.

Step 3: Form RNP Complexes (Recommended)

Be sure to use the appropriate Cas9 nuclease (e.g., Cas9 2NLS) for your cell type or application.

1. Synthego Cas9 2NLS (sold separately) has a concentration of 20 µM (20 pmol/µl) and requires no further dilution. Cas9 nuclease from other vendors can be diluted to 20 µM (20 pmol/µl) in a suitable buffer.

2. Form sgRNA:Cas9 RNP complexes in a sterile microcentrifuge tube or plate at an appropriate ratio in a volume suitable for the type of RNP delivery method to be used.

Example Protocol: Add 6 µl (180 pmol) diluted sgRNA (30 µM) and 1 µl (20 pmol) Cas9 nuclease (20 µM) to 23 µl of appropriate electroporation buffer to create a total volume of 30 µl RNP complex for each transfection of 150,000 cells.

Note: You may need to experimentally determine both the optimum sgRNA:Cas9 ratio and RNP concentration for your cell type or experiment. Synthego recommends sgRNA:Cas9 ratios between 3:1 and 9:1 for RNP complexes (nucleofection/electroporation), 1.3:1 for lipid transfection, or 1:1 for microinjection. Please visit synthego.com/resources to find recommended protocols.

3. Incubate at room temperature for 10 minutes to assemble the RNP complexes.

4. RNP complexes are stable at room temperature for up to 1 hour, at 4 °C for up to one week, or at -20 °C for up to 1 month.

Step 4: Deliver RNPs to Cells

For specific protocols, please visit synthego.com/resources. Be sure to use appropriate positive and negative controls in your CRISPR experiment.

Step 5: Inference of CRISPR Edits (ICE) Analysis

ICE is a free online tool that provides an easy quantitative assessment of genome editing using Sanger sequencing data. The software compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited pools of cells in the previous step. The ICE analysis tool, instructions and a tutorial are available at ice.synthego.com.

For more information or if you have any questions or comments, please contact us at support@synthego.com.