sgRNA
Quick Start Guide

CRISPRevolution by SYNTHEGO

For Research Purposes Only

Welcome to the CRISPRevolution

Thank you for choosing Synthego CRISPRevolution synthetic RNA for your CRISPR experiment! You have purchased world-class guide (gRNA) that offers an unbeatable combination of quality, speed, accuracy, and price.

Step 1: Rehydrate Your sgRNA

Synthego RNA oligonucleotides are dried down prior to shipping at ambient temperature. They will remain stable in this format 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: The value printed on the tube or plate represents the quantity of material present as measured by UV absorbance spectroscopy at a wavelength of 260 nm, prior to dehydration. Upon hydration, and prior to experimental use, it is best practice to verify RNA concentration using a sensitive UV absorbance spectroscopy instrument, such as a Nanodrop™. You may notice a small variation between the printed value and what you measure. Such variations are normal.

1. Briefly centrifuge tubes or plates containing oligos to ensure RNA pellets are collected at the bottom.
2. Carefully hydrate sgRNA in an appropriate nuclease-free buffer and pulse vortex for 30 seconds to ensure complete mixing.

   Recommended hydration for cell lines and primary cells: To each sgRNA (3 nmol), add 30 µl of the provided nuclease-free 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for a final concentration of 100 µM (100 pmol/µl).

   Recommended hydration for embryo 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.

   Recommended protocol: Add 6 µl of 100 µM sgRNA to 14 µl of the provided 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 (up to 6 months if not being thawed repeatedly).

Step 3: You are Now Ready to Use Your Synthetic sgRNA!

Synthego recommends forming ribonucleoprotein (RNP) complexes for your genome editing experiments in order to maximize editing efficiency and reduce off-target effects. For an example of how to form RNP complexes, proceed to Step 4.

Step 4: Form RNP Complexes (Optional)

Be sure to use the appropriate Cas9 (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) of diluted sgRNA (30 µM) and 1 µl 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 (electroporation), 1.3:1 for lipid transfection, or 1:1 for microinjection.

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 5: Deliver RNPs to Cells

Be sure to use appropriate positive and negative controls in your CRISPR experiment. For specific protocols, please visit www.synthego.com/protocols. For information about guide validation, CRISPR editing analysis tools or if you have any questions or comments, please contact us at support@synthego.com.

crRNA & tracrRNA
Quick Start Guide

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Welcome to the CRISPRevolution

Thank you for choosing Synthego CRISPRevolution Synthetic RNA for your CRISPR experiment!
You have purchased world-class guide RNA that offers an unbeatable combination of quality, speed, accuracy, and price.

Step 1: Rehydrate Your crRNA & tracrRNA

Synthego RNA oligonucleotides are dried down prior to shipping at ambient temperature. They will remain stable in this format 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: The value printed on the tube or plate represents the quantity of material present as measured by UV absorbance spectroscopy at a wavelength of 260 nm, prior to dehydration. Upon hydration, and prior to experimental use, it is best practice to verify RNA concentration using a sensitive UV absorbance spectroscopy instrument, such as a Nanodrop™. You may notice a small variation between the printed value and what you measure. Such variations are normal.

1. Briefly centrifuge tubes or plates containing oligos to ensure RNA pellets are collected at the bottom.
2. Carefully hydrate RNA in an appropriate nuclease-free buffer and pulse vortex for 30 seconds to ensure complete mixing.

   Recommended hydration for cell lines and primary cells: To each crRNA and tracrRNA (5 nmol), add 25 µl of the provided nuclease-free 1X TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for a final concentration of 200 µM (200 pmol/µl).

   Recommended hydration for embryo microinjection: It is critical to only hydrate and dilute RNA in a nuclease-free 1X Microinjection Buffer (e.g., 10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0; not provided).

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

Step 2. Anneal Your crRNA & tracrRNA

1. To make annealed gRNA, add crRNA and tracrRNA in a 2:1 ratio to a larger volume of 5x Synthego Annealing Buffer and nuclease-free water in a sterile microcentrifuge tube or plate.

   Recommended protocol: Add 12 µl of 200 µM crRNA and 6 µl of 200 µM tracrRNA to 8 µl of the provided 5x Synthego Annealing Buffer and 14 µl of nuclease-free water to make a total volume of 40 µl of 30 µM annealed gRNA (30 pmol/µl).

2. Heat to 78°C for 10 minutes on a heating block or thermocycler block, then at 37°C for 30 minutes.
3. Remove from heat and allow to cool slowly (approx. 15 minutes) to room temperature on the benchtop in order to promote stable RNA secondary structure formation. Gradual ramping on a thermocycler block can also be used.
4. Use annealed gRNA immediately or store at -20 °C for up to 1 month.

Step 3: You are Now Ready to Use Your Synthetic crRNA & tracrRNA!

Synthego recommends forming ribonucleoprotein (RNP) complexes for your genome editing experiments in order to maximize editing efficiency and reduce off-target effects. For an example of how to form RNP complexes, proceed to Step 4.

Step 4: Form RNP Complexes (Optional)

Be sure to use the appropriate Cas9 (e.g., Cas9-2NLS etc.) 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 gRNA:Cas9 RNP complexes in a sterile microcentrifuge tube or plate at an appropriate ratio in a volume suitable for the type RNP delivery method to be used.

   Example protocol: Add 6 µl (180 pmol) of annealed gRNA (30 µM) and 1 µl 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: that you may need to experimentally determine both the optimum gRNA: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 (electroporation), 1.3:1 for lipid transfection, or 1:1 for microinjection.

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 5: Deliver RNPs to Cells

Be sure to use appropriate positive and negative controls in your CRISPR experiment. For specific protocols, please visit www.synthego.com/protocols. For information about guide validation, CRISPR editing analysis tools or if you have any questions or comments, please contact us at support@synthego.com.