Synthetic sgRNA Kit

Thank you for choosing Synthego's Synthetic sgRNA Kit for your CRISPR experiment! Our world-class sgRNA offers an unbeatable combination of quality, accuracy, speed, and price. The sgRNA may be used for knockouts, knock-ins, and other CRISPR applications. This quick start guide provides a basic overview of how to prepare and use your sgRNA in a CRISPR experiment. Instructions for handling sgRNA in tube and plate formats are included.

Materials Provided

Quantity	Name	Description	Storage
1.5 nmol	Target-specific sgRNA	Shipped in 1 tube MW of an avg 100-nt sgRNA is 32 ug/nmol	-20˚C for up to up to 12 months if not repeatedly thawed.
1.5 ml*	Nuclease-free Tris-EDTA Buffer (1X TE buffer)	10 mM Tris, 1 mM EDTA (pH 8.0)	Room temperature
1.5 ml*	Nuclease-free water	-	Room temperature

*One 1.5 ml tube is provided per order (which may contain 1 or more sgRNA kits).

Additional Materials Required

Name	Description	Ordering Information
Cas9 2NLS nuclease	Wild type Cas9 from *S. pyogenes* (20 µM, 162 ug/nmol)	Synthego (available at checkout)
Controls Kit (recommended for optimization)	Includes: Positive control sgRNA (human *RELA* & human *CDC42BPB*) Negative control sgRNA, modified PCR and sequencing primers Nuclease-free Tris-EDTA Buffer Nuclease-free water Cas9 NLS nuclease from *S. pyogenes* (300 pmol, 20 µM, 162 ug/nmol)	Synthego (available at checkout)
Plate seals (for sgRNA in plates only)	Covers for multiwell plates, to be used with thermal microplate sealer	Agilent, peelable aluminum, Product #24210-001
Thermal microplate sealer (for sgRNA in plates only)	Applies seals to plates using heat	Agilent PlateLoc

Note: See selected transfection protocol for additional materials (link to protocols in Step 3).

Workflow Schematic

6 DAYS

Optimization of Transfection

Transfection Using Positive Control sgRNA

PCR & Sanger Sequencing

ICE Analysis

6 DAYS

Knockout Experiment

Transfection Using Target sgRNA

PCR & Sanger Sequencing

ICE Analysis

2–8 WEEKS

Clonal Isolation of Knockout (optional)

Limiting Dilution

Clonal Expansion

Step 1: Rehydrate the 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 12 months).

Be sure to work in an RNase-free environment.

Note: The quantity of material printed on the tube or plate is measured by UV absorbance spectroscopy at a wavelength of 260 nm, prior to dehydration. Upon rehydration, and prior to experimental use, it is best practice to verify RNA concentration using a sensitive UV absorbance spectroscopy instrument, such as a Nanodrop™. Small variations between the printed value and what you measure are normal.

sgRNA in Tubes

1. Briefly centrifuge tubes 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). The following table states the recommended amount of nuclease-free 1X TE buffer for six different starting quantities 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

Note: For microinjection: It is critical to ***only*** rehydrate 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).

Pulse vortex for 30 seconds to ensure that the sgRNA is fully resuspended. If you notice any remaining pellet or precipitate, then gently mix with a pipette or vortex until the ***sgRNA is completely dissolved***. If sgRNA does not fully dissolve, incubate at 4°C overnight.

3. Rehydrated sgRNA should be stored at -20°C. Under these conditions, sgRNA will be stable for up to 12 months. We recommend storing sgRNA at a concentration of 100 µM rather than in more dilute concentrations.

sgRNA in Plates

1. Centrifuge the 96-well plate containing the sgRNA at 4,000 rpm for 30 seconds to ensure that RNA collects at the bottom of each well.
2. Remove the seal by firmly holding the plate in place on a table and then gently pulling the seal off starting at one corner.
3. Instructions on how to rehydrate RNA in 96-well plates to a storage concentration are described below. An example dilution to a working stock is also provided.
4. Add nuclease-free 1X TE buffer to desired wells according to the appropriate sgRNA volume (see the table below). The final concentration of the sgRNA will be 100 µM (100 pmol/µl).

sgRNA (nmol)	1.5	3
Nuclease-free 1X TE buffer* (µl)	15	30

Note: 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).

1. Re-seal plates with a thermal-based heat sealer. Synthego recommends sealing plates using the Agilent PlateLoc Thermal Microplate Sealer at 160°C for 2.8 seconds.
2. Let sit overnight at 4°C to fully rehydrate dried sgRNA. Make sure the ***sgRNA is completely dissolved***.
   Note: rehydrated sgRNA may be stored at -20 °C for up to 12 months.
3. When ready to use the sgRNA, let plate warm to room temperature over 15 minutes to prevent condensation on the seal.
4. Vortex sealed plate at low speed for 30 seconds to mix.
5. Centrifuge plate at 4,000 rpm for 30 seconds.
6. Remove the seal by firmly holding the plate in place on a table and then gently pulling the seal off starting at one corner to prevent cross-contamination.
7. The sgRNA can now be used directly or diluted further to a working stock (see below).

Note: re-seal plate after each use with the thermal-based heat sealer at 160°C for 2.8 seconds.

Step 2: Dilute the sgRNA (optional)

1. Depending on the application, sgRNA may be used directly at the storage concentration (100 µM) in 1X TE buffer or be diluted to a working stock using nuclease-free water in a sterile microcentrifuge tube or plate. Check your transfection protocol for the desired sgRNA concentration.

   Working stock example calculation: To make a 30 µM sgRNA (30 pmol/µl) working stock, add 6 µl of 100 µM sgRNA to 14 µl of nuclease-free water (total volume of 20 µl).

2. Use diluted sgRNA immediately or store at -20°C for up to 3 months (or 12 months if not repeatedly thawed).

Step 3: Optimize Conditions & Transfect Cells

We highly recommend that you optimize transfection conditions in your particular cell type prior to using your target-specific sgRNA. Synthego's Controls Kit contains two positive control sgRNAs that can be used for optimization (see Additional Materials Required on pg 1). We recommend forming ribonucleoprotein (RNP) complexes for your genome editing experiments to maximize editing efficiency and reduce off-target effects.

Please visit Synthego.com/resources to find recommended transfection protocols.

Step 4: Analyze Knockout Efficiency

Synthego's Inference of CRISPR Edits (ICE) is a free online tool that provides an easy quantitative assessment of genome editing using Sanger sequence data. The software compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited pools of cells. The tool is available at ice.synthego.com.

Protocols for genomic DNA preparation, ICE analysis, and clonal isolation are available at Synthego.com/resources.

Additional Information

For an up-to-date list of all Synthego Protocols and other resources, please visit Synthego.com/resources

For technical assistance, please see www.help.synthego.com

Scientific Support Team:
Ph: 888.611.6883 Email: support@synthego.com

About Synthego

Synthego is the leading genome engineering innovation company. The company's automated, full stack genome engineering platform enables broader access to CRISPR to accelerate basic scientific discovery, uncover cures for diseases, and develop novel synthetic biology applications. Headquartered in Silicon Valley, Synthego is used by scientists from the largest global biotechnology companies and global biology universities to unlock the potential of gene editing.