SureGuide Single Guide RNAs

Product Overview

For Research Use Only. Not for use in diagnostic procedures.

About the SureGuide Single Guide RNAs

Design and Synthesis

A single guide RNA (sgRNA) is an RNA oligo that contains the sequences necessary for binding to a Cas nuclease as well as a sequence that binds a genomic target site of interest. SureGuide sgRNAs are custom designed and ordered through the Agilent CRISPR gRNA Design Software, which allows you to specify the entire sequence of your sgRNA, including the genomic target site of interest and the binding sequences for the Cas nuclease of your choice. The CRISPR gRNA Design Software also allows you to select any chemical modifications for the sgRNA and place an order. Agilent then synthesizes the SureGuide sgRNAs using a technique that polymerizes 2′-*O*-thionocarbamate-protected monomers (see **"Publication"** on page 4). The RNA is dried prior to shipment.

Consult the product insert that shipped with your SureGuide sgRNA for information pertaining to your specific sgRNA.

Storage and Handling of sgRNA

Storage Conditions

Store the dried sgRNA at –20°C until ready to be reconstituted.

Once reconstituted (see instructions in **"Reconstitute the sgRNA"** below), store the sgRNA at –80°C. To limit freeze/thaw cycles, Agilent recommends aliquotting the reconstituted sgRNA into smaller working volumes prior to storage at –80°C.

Precautions for Handling RNA

To prevent contamination of the RNA by nucleases, always wear powder-free laboratory gloves and use dedicated solutions and pipettors with nuclease-free aerosol-resistant tips. Maintain a clean work area.

Reconstituting the sgRNA

The RNA is dried prior to shipment. Before you can use the SureGuide sgRNA in an application, you must first reconstitute the dried RNA.

Materials Required

Table 1 Materials required for reconstituting the sgRNA

Material
Powder-free laboratory gloves
TE buffer, pH 7.4, nuclease-free
Microcentrifuge
Vortex mixer
Micropipettors and micropipettor tips (nuclease-free, aerosol-resistant)
Microcentrifuge tubes (0.5, 1.5, or 2.0 mL), RNase-free
Orbital shaker (optional)
Ice/ice bucket

Reconstitute the sgRNA

Follow the instructions below to reconstitute the sgRNA.

1. Before opening the cap on the tube of sgRNA, briefly spin the tube in a microcentrifuge.
2. Based on your downstream application, determine the desired concentration for your sgRNA stock.

   For example, if you plan to co-transfect the sgRNA and Cas protein into host cells as a ribonucleoprotein complex, Agilent recommends reconstituting to a stock concentration of 5 µg/µl. See **"Recommendations for using SureGuide sgRNA in transfection protocols"** on page 3 for more information.
3. Using the quantity of sgRNA stated on the product insert, calculate the quantity of TE buffer (pH 7.4) needed to make a stock solution of the desired concentration. Add that quantity of TE buffer to the tube then recap the tube.
4. To allow the sgRNA to fully dissolve, place the tube on an orbital shaker or vortex mixer for 30 minutes at room temperature.
5. Briefly spin the tube in a microcentrifuge then visually inspect the tube for the presence of any undissolved sgRNA.
   - If you see undissolved particles in the solution, briefly mix the sgRNA on a vortex mixer then inspect the tube again. If necessary, mix on vortex mixer again then reinspect.
   - If you continue to see undissolved particles after repeated mixing at room temperature, then incubate the sealed tube at 37°C for 1 minute, followed by briefly mixing on a vortex mixer. Repeat, if necessary, until the sgRNA is completely dissolved.

   Once the sgRNA is completely dissolved, use immediately or store the stock solution at –80°C. To limit freeze/thaw cycles, Agilent recommends aliquotting the sgRNA into smaller working volumes prior to storage.

   Keep the tube of sgRNA stock solution on ice when it is not in the freezer.

Quantifying the sgRNA (optional)

Once reconstituted, Agilent recommends quantifying the concentration of the sgRNA using a NanoDrop spectrophotometer.

Materials Required

Table 2 Materials required for quantifying the sgRNA

Material
Powder-free laboratory gloves
TE buffer, pH 7.4, nuclease-free
Micropipettors and micropipettor tips (nuclease-free, aerosol-resistant)
NanoDrop spectrophotometer

Quantify the sgRNA

Follow the instructions below to quantify the RNA concentration in your stock solution.

1 Measure the concentration of the stock solution on a NanoDrop spectrophotometer.

   Make sure to blank the instrument using the same buffer in which the sgRNA is reconstituted (i.e., TE buffer, pH 7.4).

2 Verify that the $A_{260/230}$ ratio is ≥ 2.0.

Recommendations for using SureGuide sgRNA in transfection protocols

Depending on your downstream applications, you may need to transfect the sgRNA into a cell line as part of an RNP (a Cas-sgRNA ribonucleoprotein complex) or co-transfect the sgRNA with Cas9 mRNA or a Cas9 plasmid. Use a transfection technique and cell line that are appropriate for your needs.

To co-deliver the Cas enzyme and sgRNA into the same host cell via RNP complex, prepare the RNP complex prior to transfection by mixing the sgRNA and the isolated Cas protein. Agilent recommends using a 2.5-fold molar excess of the sgRNA over the Cas protein in the mixture.

Example In typical electroporation/nucleofection procedures with a 20-µl transfection volume, combine 50 pmole of Cas protein (equates to ~8 µg SpyCas9) with 125 pmole of SureGuide sgRNA (equates to ~4 µg of a 100 nucleotide sgRNA). Incubate the mixture for 15 minutes at room temperature prior to transfection.

When reconstituting your SureGuide sgRNA, Agilent recommends reconstituting to a stock concentration of 5 µg/µl. This stock concentration of sgRNA allows you to keep the volume of the RNP complex to a minimum in the transfection reactions.

Notices to Purchaser

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SureGuide sgRNA ("Product") is offered for sale by Agilent ("Company") under the following limited licenses. Customer's acceptance of delivery and/or use of the Product shall constitute Customer's binding agreement to the following terms and conditions. If Customer is unwilling to accept such terms and conditions, Company is willing to accept return of the Product, prior to any use of the Product, for a full refund.

a. This product is purchased from Agilent Technologies Inc. and may contain inventions, modifications or improvements that are covered by patents owned or controlled by Agilent Technologies, Inc or its affiliates. Agilent hereby grants a limited, nontransferable right to use the sgRNA, solely as provided to Customer, together with Derivatives, only to perform Internal Research for the sole benefit of Customer and for no other purpose.

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Licensed Patent Rights

Patent information for CRISPR SureGuide chemically synthesized sgRNA can be found at:

www.agilent.com/en/product/crispr-cas/chemically-synthesized-sgrna/sureguide-chemically-synthesized-sgrna-776093/patent-crispr-sureguide-chemically-synthesized-sgrna

Publication

Dellinger DJ, Timár Z, Myerson J, Sierzchala AB, Turner J, Ferreira F, 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;133:11540–11556.

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