IG® sgRNA Synthesis Kit for Cas9 Nuclease

Manual

Catalog #	3203	3206
Volume	20 µl RXN	100 µl RXN
Package Size	10 reactions	

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description:

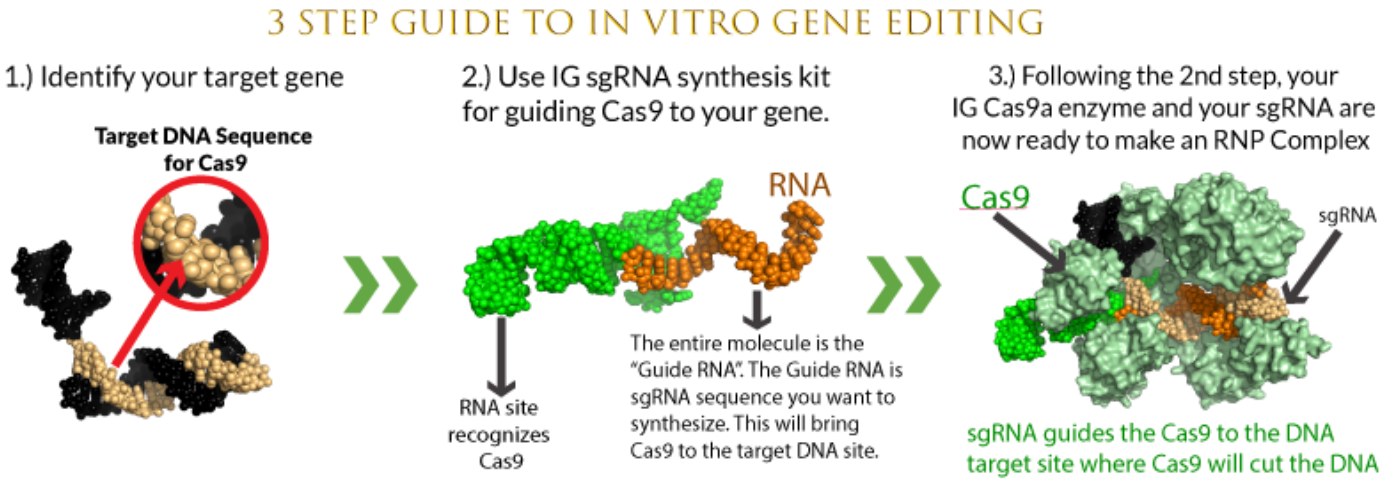
Intact Genomics is your resource for *in vitro* gene editing using Cas9 enzymes. We understand your need to generate sufficient quantities of sgRNA for *in vitro* gene editing projects. The IG® sgRNA synthesis kits are simple to use, and our kits are scalable to help you reach these goals. The IG® sgRNA synthesis kits can be purchased with or without the sgRNA purification kit.

The process of sgRNA synthesis by *in vitro* transcription (IVT) is intricate but allows for successful creation of longer sgRNAs (e.g. 100 or more nt) that cannot be easily chemically synthesized and purified at a low cost.

sgRNA synthesis can often be complicated and the maximum yield of sgRNA depends on the oligo target. Despite this complexity, it is important to use sufficient sgRNA to demonstrate the efficiency of *in vitro* DNA cutting for your gene editing. The IG® sgRNA synthesis kit solves this issue by providing at least 10ug of sgRNA per reaction (100 ul), enough for *in vitro* DNA cleavage/editing in most cases. Various competitors' kits can only claim production of a minimum of 4µg of RNA. The increased yield from the IG® sgRNA synthesis kits will enhance your chances of experimental success. This kit provides large sgRNA synthesis volumes to get more yield.

For successful sgRNA Synthesis, scientists on your team need simply to identify a DNA modification target of interest and order unique primers targeting that sequence. From there, our team provides a simple workflow to quickly complete sgRNA synthesis, so that you may progress to gene editing using IG® Cas enzymes.

Intact Genomics is with you every step of the way to complete your projects. We offer expert advice, fast ordering and delivery, and product customization.



Benefits:

IG® sgRNA Synthesis Kits are a perfect choice for a variety of sgRNA synthesis needs. Below is a sampling of some of the key benefits.

- IG® sgRNA Synthesis Kit includes everything but your targeting oligo (all buffers, enzymes, scaffolds)
- User simply needs to provide the oligo for your target—Must be 55 base pairs and generated using the template design in this manual.
- Rapid Workflow (Less than 1 hour)
- Scalable Yield (just 1 reaction for all the sgRNA you need)
- Customer support – our team is available to aid with your success.

Components and Storage:

IG® sgRNA Synthesis Kits contains the items below. Store all components at -20°C.

- IG® sgRNA Enzyme Mix (10X)
- IG® sgRNA Reaction Mix, *S. pyogenes* (4X)
- DNase I (RNase-free, 2 Units/uL)
- IG® sgRNA Control Oligo, *S. pyogenes* (1 uM)
- Dithiothreitol (DTT, 0.2 M)
- NTPs mix (25 mM)

Items needed but not provided in this kit:

- Nuclease Free Water
- Custom 55-nucleotide oligo (see instructions below to design your oligo)
- Nuclease-free pipette tips and microcentrifuge tubes

IG® sgRNA Synthesis Protocol:

Step 1: Design your custom 55-nucleotide oligo to use the IG® sgRNA synthesis kit

These steps will help to design the sequence of the target DNA oligos needed to use the kit. These oligos are not included and should be ordered and manufactured separately to use with this kit.

1. Use a target site selection webtool to find a 20 nucleotide sequence in your gene (ChopChop, for example). We will refer to your target sequence as "N_{20}".
2. To the 5' end of N_{20}, add the T7 promotor with a guanine at the 3' position. A final "G" is important for RNA transcription although it is not part of the T7 promoter. It also gets incorporated into the final sgRNA product.
   5'-TTCTAATACGACTCACTATAG(N_{20})-3'
3. The 3' end of N_{20} needs to overlap with the IG Cas9 scaffold. Add the following scaffold overlap sequence:
   5'-TTCTAATACGACTCACTATAG(N_{20})GTTTTAGAGCTAGA -3'

The custom 55-nucleotide oligo you need to order/manufacture before using the IG® sgRNA synthesis kit is:

TTCTAATACGACTCACTATAG(N_{20})GTTTTAGAGCTAGA

where "N_{20}" is YOUR 20-nucleotide target sequence that you identified in step 1.

Custom Oligo Design EXAMPLE:

We use a specific sgRNA targeted sequence as an example as below:

A. Target-specific DNA sequence for sgRNA is selected.

Example: 5' G AAGGCAATTGATCAATTCTGGG 3'

Remove these three nucleotides: the PAM sequence (NGG, which are NOT highlighted in red) is required for Cas9 recognition of the target site and is NOT part of the sgRNA sequence as BELOW:

5' GAAGGCAATTGATCAATTCT 3'

B. Add "G" to the 3' end of T7 promoter sequence highlighted in blue to ensure transcription because at least one G is necessary for efficient T7 RNA polymerase binding. Then, attach it to the above DNA sequence of the specific sgRNA BELOW:

5' TTCTAATACGACTCACTATA**G**GAAGGCAATTGATCAATTCT 3'

C. Append the 14 nucleotide-overlap DNA sequence shown in pink, the example DNA oligo of a total length of 55 bp is to be ordered. Shown BELOW:

5' TTCTAATACGACTCACTATA**G**GAAGGCAATTGATCAATTCTGTTTTAGAGCTAGA 3'

D. The sgRNA synthesis kit includes the oligo shown below. This sequence matches the *S. pyogenes* Cas9 scaffold DNA sequence:

5' AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTT

AACTTGCTATTTCTAGCTCTAAAAC 3'

When setting up the IG® sgRNA synthesis reaction, the overlapped DNA oligos are oriented as shown below:

5'TTCTAATACGACTCACTATA**G**GAAGGCAATTGATCAATTCTGTTTTAGAGCTAGA……

3'……CAAAATCTCGATCTTTATCGTTCAATTTTATTCCGA

…… 3'

TCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCACGAAAA 5'

Following completion of the incubation, the double-stranded DNA reaction product from DNA polymerase activity is shown below:

5' TTCTAATACGACTCACTATA**G**GAAGGCAATTGATCAATTCTGTTTTAGAGCTAGAAATAG

3· AAGATTATGCTGAGTGATATCCTTCCGTTAACTAGTTAAGACAAAATCTCGATCTTTATC

CAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTT 3'

GTTCAATTTTATTCCGATCAGGCAATAGTTGAACTTTTTCACCGTGGCTCAGCCACGAAAA 5'

The final example sgRNA sequence is:

5' GGAAGGCAAUUGAUCAAUUCUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCC

GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU 3'

Step 2: Utilize the IG® sgRNA synthesis kits

You've done the hard part, now let Intact Genomics make this next part easy. Simply choose whether to follow the SMALL SCALE or the PREPARATIVE SCALE table in the protocol.

1. Prewarm an incubator or water bath for microcentrifuge tubes to 37°C.
2. Thaw each component of the IG sgRNA synthesis kit and keep the unmixed kit reagents on ice.
3. Prepare a 1 µM solution of your custom oligo.
4. Choose to follow one of the two tables below. In a DNase/RNase-free 1.5 mL conical tube, mix the following IG® sgRNA synthesis kit components IN ORDER at room temperature. (CHOOSE either the "Small Scale" OR the "Preparative Scale").

Small Scale

Reagent	Amount
Nuclease-free Water	3 µL
4X IG sgRNA reaction mix	5 µL
Your 55-nucleotide custom oligo (1 µM)	5 µL
DTT (0.2 M)	1 µL
NTPs (25mM)	4 µL
IG sgRNA enzyme mix	2 µL
Total	**20 µL**

Preparative Scale

Reagent	Amount
Nuclease-free Water	15 µL
4X IG sgRNA reaction mix	25 µL
Your 55-nucleotide custom oligo (1 µM)	25 µL
DTT (0.2 M)	5 µL
NTPs (25mM)	20 µL
IG sgRNA enzyme mix	10 µL
Total	**100 µL**

5. Mix thoroughly by tapping or flicking the tube 10 times, (do not vortex) and centrifuge the reaction droplets (for less than 5 seconds) to the bottom of the tube in a microcentrifuge. This mixture is the sgRNA synthesis reaction.
6. Transfer the sgRNA mixture to the prewarmed 37°C incubator for 30 minutes. The reaction is usually complete in about 25 minutes, and there are no negative effects if left for under an hour at 37°C.
7. Transfer the reaction to ice.
8. Add IG® DNase-I (RNase-Free) and Nuclease-free Water as described in the table below that matches your choice of "Small Scale" or "Preparative Scale":

Small Scale

Reagent	Amount
Tube with your sgRNA mixture	Already in tube (20 µL)
Nuclease-free Water	30 µL
igDNase-I (RNase-free)	2 µL
Total	**50 µL**

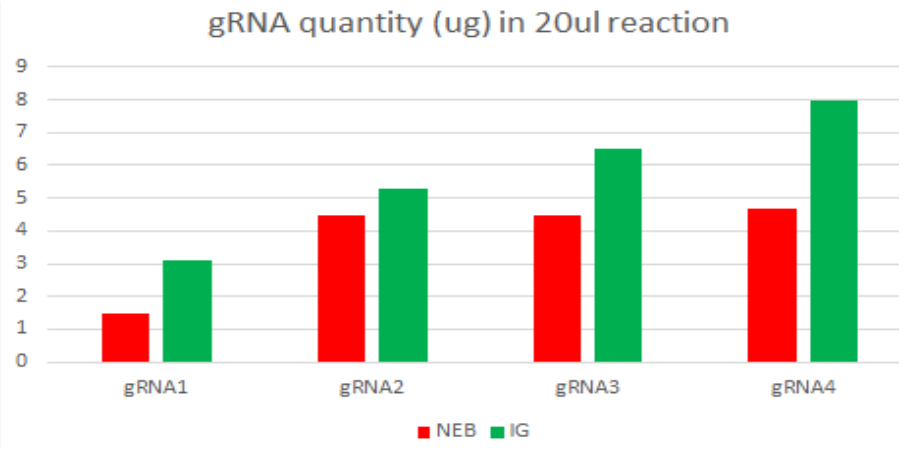
Preparative Scale

Reagent	Amount
Tube with your sgRNA mixture	Already in tube (100 µL)
Nuclease-Free Water	150 µL
igDNase-I (RNase-free)	10 µL
Total	**260 µL**

9. Mix thoroughly by tapping the tube 10 times and centrifuge (less than 5 seconds) all sample droplets to the bottom of the tube in a microcentrifuge.
10. Transfer the sgRNA mixture to the prewarmed 37°C incubator for 15 minutes.
11. For *in vitro* downstream CRISPR applications we recommend purifying the RNA using the IG® sgRNA purification kit (follow the sgRNA purification protocol) or analyzing your sgRNA by gel electrophoresis using RNase-free tips, tubes and buffers.

Comparison Data:

We compared the yields of the IG® sgRNA Synthesis Kit for Cas 9 nuclease with the New England BioLabs Inc. EnGen® sgRNA Synthesis Kit and consistently achieved greater yields of gRNA in four experiments.



	NEB	IG
gRNA1	1.5	3.1
gRNA2	4.5	5.3
gRNA3	4.5	6.5
gRNA4	4.7	8

*gRNA ug/20ul reaction

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
- Order via our distributors.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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