PROTOCOL

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Dharmacon™ Edit-R™ synthetic guide RNA resuspension protocol

This protocol is for the resuspension of Edit-R synthetic crRNA, synthetic tracrRNA, or synthetic sgRNA

1. Briefly centrifuge tubes or plates containing synthetic crRNA, tracrRNA, or sgRNA to ensure that the RNA pellet is collected at the bottom of the tube.
2. Resuspend in nuclease-free 10 mM Tris pH 7.4. for the desired final concentration using volumes listed in Table 1.
   - For example, for 10 nmol of crRNA and a 10 µM stock concentration, add 1000 µL 10 mM Tris pH 7.4. (Cat #B-006000-100)
3. Pipette the solution up and down 3–5 times, avoiding the introduction of bubbles, and securely seal tubes.
4. Place the solution on an orbital mixer/shaker for 30 minutes at room temperature.
5. Briefly centrifuge tubes containing RNA to ensure that the solution is collected at the bottom of the tube.
6. Verify the concentration using UV spectrophotometry at 260 nm. Use Beer's Law to quantify the RNAs (see FAQs for additional information). For crRNA, 1 µM = 13.5 ng/µL (on average). For tracrRNA, 1 µM = 23.8 ng/µL. For synthetic sgRNA, 1 µM = 32.3 ng/µL (on average).
7. RNA may be used immediately, or aliquoted into smaller volumes to limit the number of freeze-thaw cycles. Resuspended RNA oligonucleotides should not go through more than four to five freeze-thaw cycles to ensure RNA integrity. Synthetic crRNA, tracrRNA and sgRNA should be stored at –20 °C to –80 °C in a manual defrost or non-cycling freezer. Under these conditions, the reagents are stable for at least one year.

Technical considerations

- For efficient Edit-R crRNA:tracrRNA or Edit-R synthetic sgRNA delivery, we strongly recommend following the instructions provided by the manufacturer for the delivery method of choice (such as transfection reagent or electroporation) and taking measures to test and optimize the conditions best suited for the cell line or culture selected. Review other protocols using Dharmacon™ DharmaFECT™ transfection reagents or electroporation.
- The most commonly used method for detection of insertions and deletions (indels) in a cell population is a mismatch detection assay such as T7 Endonuclease I (T7E1, NEB). When edited cells are expanded and clonal populations isolated, the most commonly used method for confirming gene editing is Sanger sequencing.

Table 1. Recommended synthetic guide RNAresuspension volumes and concentrations.

crRNA or tracrRNA amount (nmol)	Volume (µL) of 10 mM Tris pH 7.4 to add for desired final concentration	
	200 µM Stock†	10 µM Stock
1	5	100
5	25	500
10	50	1000
20	100	2000
50	250	**Exceeds tube volume***

*When the volume exceeds the tube volume, make a 100 µM stock and dilute 10 times to obtain the 10 µM stock.
†For electroporations in 96 well format highly concentrated guide RNA stocks of 200 µM are recommended.

Frequently asked questions

Question	Answer
How do I quantify the resuspended synthetic crRNA, tracrRNA and sgRNA?	RNA is most accurately quantified by measuring its absorbance at 260 nm (A260) with a dual beam spectrophotometer.
What is the formula for spectrophotometric quantification of synthetic crRNA, tracrRNA and sgRNA?	To quantify RNA, use Beer's Law: Absorbance (260 nm) = (ε)(concentration)(path length in cm), where ε, epsilon, is the molar extinction coefficient (provided on the Product Transfer Form supplied with the crRNA or synthetic sgRNA order; the extinction coefficient for tracrRNA Cat# U-002005 is 757800). When solved for the unknown, the equation becomes: Concentration = (Absorbance, 260 nm) / [(ε)(path length in cm)]. When a standard 10 mm cuvette is used, the path length variable in this equation is 1. If a different size of cuvette is used, e.g., a 2 mm microcuvette, then the path length variable is 0.2.
Why does the calculated amount of RNA in solution differ from that on the Product Transfer Form?	Differences in instrumentation for quantifying RNA may lead to differences in apparent values. Dual beam UV-VIS spectrophotometers are recommended. Sample is too concentrated. Absorbance values are most accurate between 0.15 and 0.6 and within the linear range of a standard curve. Sample is too diluted. Measurements with dilutions of small volumes (1–1.5 µL) are more susceptible to variation. Sample may not be fully resuspended; continue shaking/mixing for an additional 15–30 minutes.
The synthetic crRNA, tracrRNA and sgRNA has been at room temperature for a week. Will the RNA still be okay?	Yes. Samples are shipped as dried pellets and are stable at room temperature for 2–4 weeks. Upon receipt, we recommend that all samples, dried or resuspended, should be stored at –20 °C to –80 °C. If degradation is a concern, the integrity of the RNA oligonucleotides can be evaluated on an analytical PAGE gel.
What is the average molecular weight of a synthetic crRNA, tracrRNA and sgRNA?	The average molecular weight (MW) of a crRNA is 13,500 g/mol. The MW of the tracrRNA is 23,759 g/mol. The average MW of a synthetic sgRNA is 32,327 g/mol.
How do I convert between nmol to µg of synthetic crRNA, tracrRNA and sgRNA?	Multiply the number of moles by the MW on the Product Transfer Form, or the average MW for your oligo. For example, 5 nmol of crRNA would be: (5 nmol)(13,500 g/mol)(mol/10^9 nmol)(10^6 µg/g) = 67.5 µg
Can I resuspend the synthetic crRNA, tracrRNA, or sgRNA in water or 1x siRNA buffer?	For resuspension of synthetic crRNA, tracrRNA, or sgRNA we recommend resuspension in nuclease-free Tris solution buffered to pH 7.4 rather than water or siRNA buffer. This will help with stability of the RNA during freeze-thaw cycles.

Read additional **Frequently Asked Questions (FAQs).**

If you have any questions, contact

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