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I. Precautions

As RNA oligonucleotides are highly susceptible to degradation by exogenous RNase that may be introduced during the handling steps, all the steps must be conducted under sterile, RNase-free conditions. RNase-free reagents, pipette tips, and tubes must be used with gloved hands while handling them.

The dried RNA oligonucleotides can be safely stored at -20°C up to 6 months

II. tracrRNA: crRNA complex formation

- Dissolve RNA oligonucleotides at your desired concentrations, e.g. 100 µM, in RNase-free water. This solution should be stored at -20°C.

Normalized amount delivered(nmol)	Resuspension buffer or Nuclease-Free Water(µl)
5	50
10	100

- Dilute each RNA oligonucleotide using an annealing buffer (e.g. 30mM HEPES, pH7.5, 100mM potassium acetate).
- Mix the crRNA and tracrRNA in equimolar concentrations in sterile microcentrifuge tube. For example, create a final duplex concentration of 25 µM using the following table.

	Volume (µl)
AccuCRISPR™-Cas9 crRNA(100µM)	10
AccuCRISPR™-Cas9 tracrRNA(100µM)	10
Nuclease-free Annealing Buffer	20
Total volume	40

- Incubate the solution for 5 minutes at 95°C, and then cool it down slowly in the room temperature. Store at 4°C or on an ice until it is ready to be used.
- crRNA:tracrRNA duplexes can be safely stored frozen at -20°C. Do not freeze-thaw more than 5 times.

III. Notice

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