

Dharmacon[™] Edit-R[™] synthetic tracrRNA, bulk amounts

This document is specific to Catalog No: U-002005-200, U-002005-500, U-002005-1000, U-002005-5000; bulk amounts of Edit-R tracrRNA.

Product Description

- Edit-R tracrRNA is a single-stranded, chemically synthesized, HPLCpurified RNA molecule based on the published, optimized *S. pyogenes* tracrRNA sequence (Jinek, 2012). It is chemically modified for improved nuclease resistance and specifically for use with Edit-R crRNA.
- Mass confirmed by MALDI-TOF mass spectrometry
- Purified by HPLC

Shipping and Storage

- Bulk Edit-R tracrRNA is shipped at ambient temperature (~23 °C) in amber, glass vials as lyophilized RNA. It will appear as a fluffy white substance. Under these conditions, it is stable for at least four weeks.
- Upon receipt, tracrRNA should be stored at -20 °C to -80 °C. Do not subject the vials to more than a 50 °C temperature change at a time. Under these conditions, the reagents are stable for at least one year.
- Edit-R tracrRNA should be resuspended in nuclease-free 10 mM Tris pH 7.4 (Dharmacon Cat #B-006000-100) to the desired final concentration.
- Upon resuspension, aliquot the tracrRNA into small volumes and store at -20 °C to -80 °C. For best results, limit freeze-thaws of each tube to no more than five events. Under these conditions, the tracrRNA is stable for at least one year.
- If glass vials will be used for short-term storage of resuspended tracrRNA, do not exceed 50% volume capacity and store at -20 °C to -80 °C. Do not subject the vials to more than a 50 °C temperature change at a time.

Handling Precautions

Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperature. Always wear gloves and maintain nuclease–free conditions. Never attempt to handle the RNA in its lyophilized form.

If you have any questions, contact

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Resuspension protocol for bulk amounts of Edit-R tracrRNA

- 1. Peel back the top of the aluminum cap to expose rubber septum.
- Using a syringe to pierce the rubber septum, resuspend tracrRNA in nuclease–free 10 mM Tris pH 7.4 (Catalog No. B006000-100). If necessary, fully remove the aluminum cap and carefully remove the stopper. Use Table 1 to determine the volume to use for the desired final concentration.
- 3. Place the vial on an orbital mixer/shaker for 30 minutes at room temperature.
- 4. Verify the concentration using UV spectrophotometry at 260 nm.
- 5. 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 RNA 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.

Table 1. Resuspension volumes for bulk Edit-R tracrRNA

Edit-R tracrRNA Cat. No.	Amount of tracrRNA	Volume of Tris Buffer for 100 μM Stock
U-002005-200	200 nmol	2 mL
U-002005-500	500 nmol	5 mL
U-002005-1000	1000 nmol	10 mL

Technical Considerations

- For addition of tracrRNA to the wells of an Edit-R crRNA Library, follow the protocol found in the Edit-R arrayed crRNA libraries guide.
- For efficient Edit-R crRNA:tracrRNA delivery, we recommend <u>DharmaFECT™ transfection reagents</u> or electroporation/nucleofection (for protocols click <u>here</u>).