

DharmaconTM Edit-RTM Lethal control crRNA

Catalog No: U-006000-01-xx and U-006000-02-xx

Edit-R Lethal Control crRNA #1 and #2 were developed to assist researchers in optimization of crRNA delivery conditions and monitoring transfection efficiency during screening. These crRNA controls are designed to kill cells by targeting thousands of locations in the genome at once, causing double-strand breaks which have been shown to result in apoptosis^{1,2}. Since the observed cell death is Cas9- and guide RNA dependent, the controls can be used to monitor both Cas9 functionality and crRNA delivery efficiency. The cell death phenotype is easily assessed by visual inspection, staining using a standard viability indicator (trypan blue or propidium iodide), or a metabolic indicator assay (resazurin). Furthermore, this cell death phenotype can develop in as few as 48 hours post-delivery, allowing for assessment of successful delivery before downstream assays need to be performed.

Product description

- Edit-R crRNA is a chemically synthesized RNA, that contains 20 nucleotides identical to multiple genomic DNA target sites followed by the required *S. pyogenes* repeat sequence that interacts with the tracrRNA, which is required for use with synthetic crRNA.
- Chemical modifications are applied to all Edit-R catalog crRNAs to resist degradation by nucleases and improve performance in applications using co-delivery with DNA-free Cas9 formats.
- Mass confirmed by MALDI-TOF mass spectrometry

Table 1. Edit-R Lethal Control crRNA.

Item description	Molecular Weight (g/mol)	Cat. #
Edit-R Lethal positive control #1	13,359.9	U-006000-01-05, -20, -50
Edit-R Lethal positive control #2	13,518.0	U-006000-02-05, -20, -50

Shipping and storage

- Edit-R Lethal Control crRNA reagents are shipped as dried pellets at room temperature (23 °C). Under these conditions, they are stable for at least four weeks.
- Upon receipt, Edit-R crRNA lethal positive control reagents should be stored at -20 °C to -80 °C. Under these conditions, they are stable for at least one year.

Resuspension

- Edit-R Lethal Control crRNA reagents should be resuspended in nuclease-free 10 mM Tris pH 7.4 (Dharmacon [Cat # B-006000-100](#)) to the desired final concentration (please see the full protocol available [here](#)).
- Upon resuspension, aliquot the crRNA 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 RNA is stable for at least one year.

Table 2. Recommended synthetic guide RNA resuspension 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
5	25	500
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 electroporation in 96 well format, highly concentrated guide RNA stocks of 200 µM are recommended.

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 when handling the oligonucleotides.

References

1. Pandey, M. and Raghavan, S.C.: DNA double-strand break repair in mammals; *J Radiat Cancer Res* **2017**; 8:93-7 DOI: 10.4103/jrcr.jrcr_18_17
2. Morgens, D.W; Wainberg, M. *et al*; Genome-scale measurement of off-target activity using Cas9 toxicity in high-throughput screens; *Nature Communications* **2017**; 8: 15178 DOI: 10.1038/ncomms15178 (2017)

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