

RNAiBoost™ Reagent Kit

CATALOG NUMBER: RNAI-201 (100 reactions in 35 mm dish/6-well)

STORAGE: -20°C. Avoid multiple freeze/thaws by aliquoting.

Components

1. RNAiBoost™ Reagent A (100X) (Part No. RNAI-20101): Two sterile tubes, 1000 µL each
2. RNAiBoost™ Reagent B (100X) (Part No. RNAI-20102): Two sterile tubes, 1000 µL each

Background

RNAi is a well-conserved mechanism that uses small noncoding RNAs to silence gene expression post-transcriptionally. It has become a mainstream molecular tool for assessing the functions of genes in mammalian cells. RNAi operates via two post-transcriptional mechanisms: targeted mRNA degradation by siRNA and suppression of translation/degradation by miRNA.

Although siRNA interacts selectively with a single target sequence within mRNA resulting sequence-specific mRNA degradation and inhibition of protein synthesis, RNAi-based analyses are often complicated by false positive and negative hits due to off-target effects and interferon response, which can be attributed at least in part to the use of high concentrations of siRNA. Lowering the amounts of siRNAs and shRNAs can effectively and expediently mitigate the off-target effect and interferon response. However, in RNAi experiments, lowering the concentration of siRNA is often accompanied by a lower knockdown efficiency.

Cell Biolabs' RNAiBoost™ Reagent Kit is a proprietary formulation that provides the following advantages:

- Enhances gene knockdown efficiency
- Reduces the concentrations of siRNA needed for the same RNAi knockdown efficiency
- Promotes the processing of miRNA precursors
- Ideal for RNAi introduced through siRNA duplex, shRNA or miRNA precursor
- Suitable for both adherent and suspension cells
- Nontoxic

Protocol

The following protocol is written for adherent cells in a 6-well plate or 35 mm culture dish. Please refer to Table 1 for the appropriate dispensing volumes for other plate and dish formats.

Culture Dish	96-well	24-well	12-well	6-well or 35 mm	60 mm	10 cm
RNAiBoost™ Reagent A (100X) (μL)	1	5	10	20	50	100
RNAiBoost™ Reagent B (100X) (μL)	1	5	10	20	50	100
Complete Culture Media (μL)	100	500	1000	2000	5000	10000

Table 1: Dispensing Volumes for Different Plate / Dish Formats

1. The day before transfection or transduction, trypsinize and count the cells, plating $1-4 \times 10^5$ cells in 2.0 mL complete culture medium per well of a 6-well plate. Incubate cells at 37°C overnight to ensure firm attachment.
2. Initiate RNAi by transfecting or transducing cells with desired siRNA, shRNA or miRNA precursor.
3. 4-8 hr after transfection or transduction, warm the RNAiBoost™ Reagents for at least 10 minutes at room temperature and add RNAi Enhancer Reagent A (100X) and B (100X) to cells to achieve a final concentration of 1X each.
Note: When changing the medium is required, RNAi Enhancer Reagent A and B (1X each) should always be included.
4. Monitor gene silencing after an appropriate time.

References

1. Hannon, G. J. (2002) *Nature* **418**: 244-251.
2. Bartel, D. P. (2004) *Cell* **116**: 281-297.
3. Lau, N. C., Lim, L. P., Weinstein, E. G., and Bartel, D. P. (2001) *Science* **294**, 858-862
4. Zamore, P. D. and Haley, B. (2005) *Science* **309**: 1519-1524.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

This product is for RESEARCH USE ONLY; not for use in diagnostic procedures.

Contact Information

Cell Biolabs, Inc.

7758 Arjons Drive

San Diego, CA 92126

Worldwide: +1 858-271-6500

USA Toll-Free: 1-888-CBL-0505

E-mail: tech@cellbiolabs.com

www.cellbiolabs.com

©2008: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.