



Dec 03, 2024

siRNA-mediated Knockdown – RAW 264.7

 [The Journal of Cell Biology](#)

DOI

dx.doi.org/10.17504/protocols.io.4r3l29owjv1y/v1

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DOI: <https://dx.doi.org/10.17504/protocols.io.4r3l29owjv1y/v1>

Protocol Citation: Amanda Bentley-DeSousa, Shawn Ferguson 2024. siRNA-mediated Knockdown – RAW 264.7. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.4r3l29owjv1y/v1>

**Manuscript citation:**

<https://doi.org/10.1101/2023.10.31.564602>

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Protocol status: Working

We use this protocol and it's working

Created: December 02, 2024

Last Modified: December 03, 2024

Protocol Integer ID: 113419

Keywords: RAW264.7, siRNA knockdown, knockdowns in raw, mediated knockdown, raw

Funders Acknowledgements:

Aligning Science Across Parkinson's Disease

Grant ID: ASAP-000580

Abstract

This protocol details steps taken to perform siRNA-mediated knockdowns in RAW 264.7 cells.

Materials

DMEM (Thermo Fisher Scientific, 11965-092)

FBS (Thermo Fisher Scientific, 16140-071)

Penicillin/Streptomycin (Thermo Fisher Scientific, 15140122)

CellStripper (Corning, 356230)

Lipofectamine RNAiMAX (Invitrogen, 2448190)

Opti-Mem (Thermo Fisher Scientific, 31985062)

Troubleshooting



Day 0

- 1 Plate 2.5×10^5 RAW 264.7 cells per well in a 6-well dish.









Day 1

30m

- 2 Transfect cells using Lipofectamine RNAiMAX and gene specific siRNAs.

2.1 RNAiMAX protocol

30m

In Tube 1 combine  112.5 μL Opti-MEM and  4.5 μL Lipofectamine RNAiMAX then incubate for  00:05:00 . In Tube 2 combine  132 μL Opti-MEM and  2 μL siRNA (final concentration of 100 nM) then incubate for  00:05:00 . Combine Tube 1 and Tube 2. Incubate for  00:20:00 . Add drop-wise to 1 well containing  1.75 mL of media.

Day 3

- 3 Change the media and perform subsequent experiments.