invitrogen™

Lipofectamine™ RNAiMAX

Cat. No. 13778-075 **Size: 0.75 ml**
Cat. No. 13778-150 **Size: 1.5 ml**
Store at +4°C (do not freeze)

Description

Lipofectamine™ RNAiMAX is a proprietary formulation specifically developed for the transfection of siRNA and Stealth™ RNAi duplexes into eukaryotic cells. Lipofectamine™ RNAiMAX provides the following advantages:

- High transfection efficiencies in many cell types to minimize background expression from untransfected cells and maximize knockdown.
- Minimal cytotoxicity to reduce non-specific effects and cellular stress.
- Generally requires low concentrations of RNAi duplexes to obtain high knockdown levels, further minimizing non-specific effects.
- A broad peak of optimal transfection activity with minimal cytotoxicity, allowing achievement of high knockdown levels despite differences in cell density, minor pipetting inaccuracies, and other variations.

Important Guidelines for Transfection

- Reverse transfection (page 2) and forward transfection (page 3) protocols can be used for most cell lines tested. Cell-type specific transfection protocols are available at www.invitrogen.com/RNAi or through Technical Service.
- We recommend Opti-MEM® I Reduced Serum Medium (Cat. No. 31985-062) to dilute RNAi duplexes and Lipofectamine™ RNAiMAX before complexing.
- **Do not** add antibiotics to media during transfection as this causes cell death.
- Test serum-free media for compatibility with Lipofectamine™ RNAiMAX.
- We recommend the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo for **Assessing Transfection Efficiency** (see page 2).
- Use 10 nM RNAi duplex and indicated procedure as a starting point; optimize transfections as described in **Optimizing Transfections** (page 3).

Quality Control

Lipofectamine™ RNAiMAX is tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, for absence of RNAse activity, and functionally by transfection of Stealth™ RNAi and appropriate controls into a reporter cell line.

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For technical support, contact tech_service@invitrogen.com.

Reverse Transfection

Use this procedure to reverse transfect Stealth™ RNAi or siRNA into mammalian cells in a **24-well format** (for other formats, see **Scaling Up or Down Transfections**, page 4). In reverse transfections, the complexes are prepared inside the wells, after which cells and medium are added. Reverse transfections are faster to perform than forward transfections, and are the method of choice for high-throughput transfection. Optimize transfections as described in **Optimizing Transfections** (page 3), especially if transfecting a mammalian cell line for the first time. All amounts and volumes are given on a per well basis.

1. **For each well to be transfected,** prepare RNAi duplex-Lipofectamine™ RNAiMAX complexes as follows.
   a. Dilute 6 pmol RNAi duplex in 100 µl Opti-MEM® I Medium without serum in the well of the tissue culture plate. Mix gently.
   b. Mix Lipofectamine™ RNAiMAX gently before use, then add 1 µl Lipofectamine™ RNAiMAX to each well containing the diluted RNAi molecules. Mix gently and incubate for 10-20 minutes at room temperature.
2. Dilute cells in complete growth medium **without antibiotics** so that 500 µl contains the appropriate number of cells to give 30-50% confluence 24 hours after plating. Use 20,000-50,000 cells/well for suspension cells.
3. To each well with RNAi duplex - Lipofectamine™ RNAiMAX complexes, add 500 µl of the diluted cells. This gives a final volume of 600 µl and a final RNA concentration of 10 nM. Mix gently by rocking the plate back and forth.
4. Incubate the cells 24-72 hours at 37°C in a CO_2 incubator until you are ready to assay for gene knockdown.

Assessing Transfection Efficiency

To assess transfection efficiency, we recommend using the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo (Cat. No. 14750-100; available through www.invitrogen.com), which is a red-labeled dsRNA oligomer designed for use with Invitrogen's Stealth™ RNAi, standard unmodified siRNA, or purified Dicer-generated siRNA. The BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo facilitates assessment and optimization of dsRNA oligonucleotides delivery into mammalian cells, and allows strong, easy fluorescence-based detection. See the BLOCK-iT™ Alexa Fluor® Red Fluorescent Oligo manual for more information.

Note: The related BLOCK-iT™ Fluorescent Oligo (Cat. No. 2013) is optimized for use with Lipofectamine™ 2000, and is not recommended for Lipofectamine™ RNAiMAX.

Forward Transfection

Use this procedure to forward transfect Stealth™ RNAi or siRNA into mammalian cells in a **24-well format** (for other formats, see **Scaling Up or Down Transfections**, page 4). In forward transfections, cells are plated in the wells, and the transfection mix is generally prepared and added the next day. Optimize transfections as described in **Optimizing Transfections** (page 3), especially if transfecting a mammalian cell line for the first time. All amounts and volumes are given on a per well basis.

Note: For some cell lines (e.g. MCF-7 or HepG2), we recommend reverse transfections.

1. One day before transfection, plate cells in 500 µl of growth medium without antibiotics such that they will be **30-50% confluent** at the time of transfection.
2. **For each well to be transfected**, prepare RNAi duplex-Lipofectamine™ RNAiMAX complexes as follows:
   a. Dilute 6 pmol RNAi duplex in 50 µl Opti-MEM® I Reduced Serum Medium without serum. Mix gently.
   b. Mix Lipofectamine™ RNAiMAX gently before use, then dilute 1 µl in 50 µl Opti-MEM® I Reduced Serum Medium. Mix gently.
   c. Combine the diluted RNAi duplex with the diluted Lipofectamine™ RNAiMAX. Mix gently and incubate for 10-20 minutes at room temperature.
3. Add the RNAi duplex-Lipofectamine™ RNAiMAX complexes to each well containing cells. This gives a final volume of 600 µl and a final RNA concentration of 10 nM. Mix gently by rocking the plate back and forth.
4. Incubate the cells 24-48 hours at 37°C in a CO_2 incubator until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours.

Optimizing Transfections

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNAi duplex and Lipofectamine™ RNAiMAX concentrations. Test 0.6-30 pmol RNAi duplex (final concentration 1-50 nM) and 0.5-1.5 µl Lipofectamine™ RNAiMAX for 24-well format. For extended time course experiments (> 72 hours), consider a cell density that is 10-20% confluent 24 hours after plating.

Note: The concentration of RNAi duplex required will vary depending on the efficacy of the duplex.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine™ RNAiMAX, RNAi duplex, cells, and medium used in proportion to the relative surface area, as shown in the table.

Culture vessel	Rel. surf. area[1]	Vol. of plating medium	Dilution medium reverse transfection	Dilution medium forward transfection	RNAi (pmol)	RNAi (nM)	Lipofectamine™ RNAiMAX[2]
96-well	0.2	100 µl	20 µl	2 x 10 µl	0.12-6	1-50	0.1-0.3 µl
48-well	0.4	200 µl	40 µl	2 x 20 µl	0.24-12	1-50	0.2-0.6 µl
24-well	1	500 µl	100 µl	2 x 50 µl	0.6-30	1-50	0.5-1.5 µl
6-well	5	2.5 ml	500 µl	2 x 250 µl	3-150	1-50	2.5-7.5 µl
60 mm	10	5 ml	1 ml	2 x 500 µl	6-300	1-50	5-15 µl
100 mm	30	10 ml	2 ml	2 x 1 ml	12-600	1-50	15-35 µl

[1] Surface areas may vary depending on the manufacturer.

[2] If the volume of Lipofectamine™ RNAiMAX is too small to dispense accurately, and you cannot pool dilutions, predilute Lipofectamine™ RNAiMAX 10-fold in Opti-MEM® I Reduced Serum Medium, and dispense a 10-fold higher amount (should be at least 1.0 µl per well). Discard any unused diluted Lipofectamine™ RNAiMAX.

Cotransfecting DNA and RNA using Lipofectamine™ RNAiMAX

For cotransfections of plasmid DNA and Stealth™ RNAi or siRNA into mammalian cells, we recommend using Lipofectamine™ 2000 (Catalog no. 11668-027), which is superior for plasmid transfections. If you want to use Lipofectamine™ RNAiMAX for your cotransfections, perform a reverse transfection as described on page 2 with the following modifications:

1a: Add 20 ng (for 24-well format) of plasmid DNA to the diluted RNAi duplex.

2: Add cells such that they will be 80-100% confluent 24 hours after plating.

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