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Oligofectamine™ Reagent

Cat. nos. 12252-011 **Size** 1 mL **Store at 4°C (do not freeze)**

Part no. 12252.pps MAN0001065 **Rev. Date** 27 July 2011

Description

Oligofectamine™ Reagent is a proprietary formulation for transfecting oligonucleotides (1) and short interfering RNA (siRNA) (2, 3) into eukaryotic cells. See the Cell Lines database at www.invitrogen.com for a list of successfully transfected cell types. The reagent formulation has been changed to enhance its stability at cold temperatures (≤4°C) while continuing to provide the highest specific activity and lowest non-specific effects on cell growth. Performance may be enhanced in some assays.

Important Guidelines for Transfection

- The **Transfection Procedure** on page 2 is used to transfect cells with oligonucleotides. Refer to www.invitrogen.com/sitransfection for siRNA.
- Use an initial oligonucleotide concentration of 200 nM for transfection. Optimal oligonucleotide concentrations may range from 50–250 nM. Always include a control oligonucleotide in the experiment to assess non-specific using the recommended amounts of oligonucleotide and Oligofectamine™ Reagent (see page 3).
  Note: We recommend using Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) to dilute Oligofectamine™ Reagent and oligonucleotide.
- Prepare complexes -MEM® I Reduced Serum Medium (Cat. no. 31985-062) to dilute Oligofectamine™ Reagent and oligonucleotide.
- Transfect cells at 30–50% confluence. Maintain the same seeding conditions between experiments. Use cells within 20 passages of optimization.
- *Do not* add antibiotics to media during transfection; this causes cell death.
- For optimal results, perform transfection in medium without serum. You may test transfection in the presence of serum, if desired. Test any serum-free medium for compatibility with Oligofectamine™ Reagent.

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Transfection Procedure

Use the following procedure to transfect *adherent* mammalian cells in a *96-well format*. For other formats, see **Scaling Up Transfections** on page 3. All amounts and volumes are given on a per-well basis.

1. One day before transfection, plate cells in 100 µL of growth medium without antibiotics so that cells will be 30–50% confluent at the time of transfection.
2. *For each transfection sample,* prepare complexes as follows:
   a. Dilute 1 µL of a 20 µM stock oligonucleotide in 16 µL of Opti-MEM® I Reduced Serum Medium (or other medium) without serum to a final volume of 17 µL. Mix gently.
   b. Mix Oligofectamine™ Reagent gently before use, then dilute 0.4–0.8 µL in Opti-MEM® I Medium (or other medium) without serum to a final volume of 3 µL. Mix gently and incubate for 5–10 minutes at room temperature.
   c. Combine the diluted oligonucleotide with diluted Oligofectamine™ Reagent (total volume = 20 µL). Mix gently and incubate for 15–20 minutes at room temperature (the solution may appear cloudy).
3. While complexes are forming, remove the growth medium from the cells and wash once with medium without serum. Add 80 µL of medium without serum to each well containing cells.
4. Mix the 20 µL of complexes (from step 2c of this procedure) gently, and add to the cells.
5. Incubate the cells at 37°C in a CO_2 incubator for 4 hours.
6. Add 50 µL of growth medium containing 3X the normal concentration of serum without removing the transfection mixture.
7. Assay for gene activity at 24–72 hours post-transfection or as appropriate for your cell type and target.

Scaling Up Transfections

To transfect cells in different tissue culture formats, vary the amounts of Oligofectamine™ Reagent, oligonucleotide, cells, and medium used in proportion to the relative surface area, as shown in the following table and given on a per-well basis. See page 1 for additional recommendations about the amount of oligonucleotide to transfect. For highest transfection efficiency, we recommend that you optimize the transfection conditions.

Culture vessel	Relative surf. area vs. 96-well	Oligo (µL of 20 µM stock) & dilution vol. (µL)	Oligofectamine™ Reagent (µL) & final dilution vol. (µL)	Plating medium vol.	Total vol. per well	Added vol. medium with 3X serum
96-well	1X	1 µL in 16 µL	0.4–0.8 µL to 3 µL	80 µL	100 µL	50 µL
24-well	5X	2.5 µL in 40 µL	1–2 µL to 7.5 µL	200 µL	250 µL	125 µL
12-well	10X	5 µL in 85 µL	1–3 µL to 10 µL	400 µL	500 µL	250 µL
6-well	25X	10 µL in 175 µL	2–4 µL to 15 µL	800 µL	1 mL	500 µL

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, we recommend optimizing the transfection conditions by varying cell density as well as oligonucleotide and Oligofectamine™ Reagent concentrations.

References

1. Li, Y., *et al.* (2002) *J. Biol. Chem. 277*, 11352.
2. Elbashir, S.M., *et al.* (2001) *Nature 411*, 494.
3. Harborth, J., *et al.* (2001) *J. Cell Sci. 114*, 4557.

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