Invitrogen
life technologies

Oligofectamine™ Reagent

Cat. No.: 12252-011 **Size: 1 ml**

Store at 4°C.

DO NOT FREEZE. MIX GENTLY BEFORE USE.

Stable for 12 months if stored at 4°C and tightly capped.

Description:
Oligofectamine™ Reagent (1) is a proprietary formulation that is suitable for the transfection of oligonucleotides into eukaryotic cells. 1 ml is sufficient for 1,250-2,500 transfections in 96-well plates, or 500-1,000 transfections in 24-well plates. Oligofectamine™ Reagent interacts spontaneously with oligonucleotides to form transfection complexes. Oligofectamine™ Reagent has been used successfully for the transfection of a wide variety of cultured eukaryotic cells including: NIH3T3, CHO, K562, HEK293, HeLaS3, and HeLa. Oligofectamine™ Reagent has been found to have the highest specific activity and lowest non-specific effect on cell growth using c-myc targeted antisense and scrambled phosphorothioate-modified oligonucleotides as compared with other commercially available transfection reagents.

Transfection Optimization:
The most important aspect of successful transfection of oligonucleotides is the careful optimization of transfection conditions for each cell type and target. Invitrogen recommends that optimal plating cell density, amount of Oligofectamine™ Reagent, and oligo concentration are the parameters to be determined for each cell line. Cell density must be optimized and kept consistent to obtain reproducible results. It is recommended that cells be passaged less than 20 times to maintain reproducible results. Cell density should be optimized first using a constant amount of oligonucleotide and Oligofectamine™ Reagent. For plating in 96-well plates, a starting range from 0 to 100,000 cells per well is recommended. For this optimization, an oligonucleotide concentration of 200 nanomolar (nM) and an amount of 0.6 µl of Oligofectamine™ Reagent per well should be used.

The amount of Oligofectamine™ Reagent can be optimized next, using a constant amount of oligonucleotide. Invitrogen recommends starting with 200 nM oligonucleotide. With this parameter held constant, vary the amount of Oligofectamine™ Reagent to determine the optimal amount (usually 0.4 to 0.8 µl per well in 96-well plates). The concentration of oligonucleotide can also be optimized by holding the cell plating density and the amount of Oligofectamine™ Reagent constant. A starting range of 50 to 250 nM oligonucleotide is recommended. An oligo concentration greater than 250 nM may result in non-specific effects. These conditions are recommended as guidelines only.

Note: It is absolutely critical to have a control oligonucleotide to be able to determine any non-specific effects. This oligonucleotide can be a "scrambled" oligonucleotide (same length and base-composition in a random order) or a "sense" oligonucleotide if the target is mRNA.

For most cell lines tested, transfection in the absence of serum was optimal. For HeLa cells, high level transfection required the presence of serum in the medium during transfection. This option should be tested if optimization does not yield high-level transfection.

Quality Control:
1. Oligofectamine™ Reagent is tested for the absence of bacterial and fungal contamination using blood agar plates and fluid thioglycollate medium.
2. A functional assay using Oligofectamine™ Reagent involves the transfection of subconfluent HeLa (ATCC CCL 2) cells with c-myc targeted antisense and scrambled phosphorothioate-modified oligonucleotides. The cell extracts are assayed for cell proliferation using AlamarBlue™ Reagent (2).

Protocols:
Transfection of Oligonucleotides into Adherent Cells:
The following procedure suggests starting points that should yield high-efficiency transfections in most adherent cells (see Table I for volumes):
The day before transfection, trypsinize and count the cells, plating them so that they are 30-50% confluent the day of transfection. At the time of plating and during transfection, avoid antibiotics – this helps cell growth and allows transfection without rinsing the cells if they are to be transfected in the presence of serum.
1. Incubate the cells at 37°C in a humidified 5% CO_2 incubator until they reach the desired confluency. This will usually take 18-24 h, but the time will vary among cell types. **Note:** For the above cells evaluated, 30-50% confluency is optimal. However, for other cell lines or targets, optimal cell density may vary. Transfection efficiency of oligonucleotides and response to target is much more sensitive to cell density than with plasmid DNA. It is important to maintain a standard seeding protocol from experiment to experiment and to use cells within 20 passages of optimization.
2. Prepare the following oligo dilutions in microcentrifuge tubes:
   For each well in a 96-well transfection, dilute 1 µl of a 20 µM stock oligonucleotide into 16 µl medium without serum to give a 200 nM oligo concentration (based on 100 µl total volume on cells). Opti-MEM® I Reduced Serum Medium gives optimal results.
   Note: Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test media for compatibility with transfection reagent before use.

3. Prepare the following transfection reagent dilutions in microcentrifuge tubes:
   For each well in a 96-well transfection, dilute 0.4 to 0.8 µl of Oligofectamine™ Reagent into medium without serum for a final volume of 3 µl. Allow diluted reagent to sit for 5-10 min. (When transfecting different sized tissue culture plates, change the amounts of oligonucleotide, Oligofectamine™ Reagent, medium and cell density in proportion to Table 1).
4. Add diluted Oligofectamine™ Reagent to diluted oligonucleotide, mix gently, and incubate at room temperature for 15-20 min.
   Note: The solution may appear cloudy, however this will not impede the transfection.
5. Wash cells once with medium without serum.
6. For each 96-well transfection, add 80 µl medium without serum to each well containing cells.
   Note: Do not add antibacterial agents to medium during transfection. For most cell lines and targets, transfection without serum is optimal. However, if desirable for the health of the cells or to achieve higher levels of activity, transfection can be tested in normal growth media containing serum.
7. Mix gently and overlay the 20 µl of complexes onto the cells.
8. Incubate the cells for 4 h at 37°C in a CO_2 incubator.
9. Following incubation, add growth medium containing 3 times the normal concentration of serum without removing the transfection mixture.
10. Assay cell extracts for gene activity at 24-72 h post transfection.
    Note: Response time to antisense oligonucleotide is dependent on target, target location, and cell type.

Table 1: Recommended starting conditions for transfection using Oligofectamine™ Reagent. Further optimization may be necessary.

	Oligonucleotide (20 µM stock)	**Opti-MEM® I**	**Amount of Oligofectamine™ Reagent**	**Oligofectamine™ Diluted in Opti-MEM® I - Final Volume**	*Serum-Free Medium on Cells*	**Transfection Volume**	**Volume of Growth Medium with 3X serum**
Protocol Steps:	**Step 2**	**Step 2**	**Step 3**	**Step 3**	**Step 7**	**Step 8**	**Step 10**
Culture Vessel:							
96-well	1 µl	16 µl	0.4-0.8 µl	3 µl	80 µl	100 µl	50 µl
24-well	2.5 µl	40 µl	1-2 µl	7.5 µl	200 µl	250 µl	125 µl
12-well	5 µl	85 µl	1-3 µl	10 µl	400 µl	500 µl	250 µl
6-well	10 µl	175 µl	2-4 µl	15 µl	800 µl	1000 µl	500 µl

Note:
Some serum-free media formulations can inhibit cationic lipid-mediated transfection. Test any new serum-free formulation for compatibility with the transfection reagent prior to use. Media formulations that have been found to inhibit transfections are: CD 293 Medium, 293 SFM II, and VP-SFM.

References:
1. Ogilvie, M., Butash, K., and Fox, D. (2000) *Antisense & Nucleic Acid Drug Dev.*, in preparation.
2. Fields, R.D., and Lancaster, M.V. (1993) *American Biotechnology Laboratory 11 (4),* 48.

For references and protocols pertaining to transfection of your cell type, please see our web site at http://www.invitrogen.com/transfection/celltypes/