Transfecting siRNA into HeLa Cells Using Oligofectamine™

Introduction

Oligofectamine™ has been used successfully to transfect short interfering RNAs (siRNA) into HeLa cells for RNA interference (RNAi) studies (Elbashir *et al.*, 2001; Harborth *et al.*, 2001). This reference provides general guidelines and a procedure to transfect siRNA into HeLa cells using Oligofectamine™. The suggested transfection conditions are provided as a starting point. If you are using HeLa cells or another mammalian cell line, we recommend optimizing transfection conditions to obtain the best results for your target gene and siRNA.

Factors Affecting Gene Knockdown Levels

A number of factors can influence the degree to which expression of a gene of interest is reduced (*i.e.* gene knockdown) in an RNAi experiment including:

- Transfection efficiency
- Transcription rate of the gene of interest
- Protein stability
- Efficacy of the particular siRNA sequence chosen
- Growth characteristics of your mammalian cell line

Take these factors into account when designing your transfection and RNAi experiments.

General Guidelines

Follow these general guidelines when performing siRNA transfection into HeLa cells using Oligofectamine™:

1. **Transfect cells when they are 30-50% confluent.**
2. **Do not add antibiotics** to media during transfection as this will cause cell death.
3. For optimal results, use Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-062) to dilute Oligofectamine™ prior to complexing with siRNA.

Materials Needed

Have the following reagents on hand before beginning:

- HeLa cells or other mammalian cell line of interest (make sure that cells are healthy and greater than 90% viable before transfection)
- siRNA of interest (20 pmol/µl)
- Oligofectamine™ (store at +4°C until use)
- Opti-MEM® I Reduced Serum Medium (Invitrogen, Catalog no. 31985-062; pre-warmed)
- 24-well tissue culture plates and other tissue culture supplies

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Transfecting siRNA into HeLa Cells Using Oligofectamine™, continued

Suggested Conditions

To transfect HeLa cells in a 24-well format, use the following conditions as a starting point. **Note:** In RNAi studies using these conditions, >80% knockdown of an endogenous gene was observed within 48 hours after transfection.

- **Cell density:** 3 x 10^4 cells per well (cells will be about 50% confluent at the time of transfection)
- **Amount of Oligofectamine™:** 3 µl
- **Amount of siRNA of interest:** 60 pmol (20pmol/µl)

Transfection Procedure

Use the following procedure to transfect HeLa cells in a **24-well format**. If you wish to transfect cells in other tissue culture formats, you will need to determine the optimal conditions to use for your mammalian cell line.

1. One day before transfection, plate HeLa cells in 0.5 ml of growth medium without antibiotics so that they will be 50% confluent at the time of transfection.
2. **For each transfection sample**, prepare siRNA:Oligofectamine™ complexes as follows:
   a. Dilute 60 pmol of siRNA in 50 µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.
   b. Mix Oligofectamine™ gently before use, then dilute 3 µl in 12 µl of Opti-MEM® I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature.
   c. After the 5 minute incubation, combine the diluted siRNA with the diluted Oligofectamine™ (total volume is 68 µl). Mix gently and incubate for 20 minutes at room temperature to allow the siRNA:Oligofectamine™ complexes to form.
3. Add the 68 µl of siRNA:Oligofectamine™ complexes to each well. Mix gently by rocking the plate back and forth.
4. Incubate the cells at 37°C in a CO_2 incubator for 24-72 hours until they are ready to assay for gene knockdown. It is generally not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.

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References

Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs Mediate RNA Interference in Cultured Mammalian Cells. Nature *411*, 494-498.

Harborth, J., Elbashir, S. M., Bechert, K., Tuschl, T., and Weber, K. (2001). Identification of Essential Genes in Cultured Mammalian Cells Using Small Interfering RNAs. J. Cell Science *114*, 4557-4565.