Thermo Scientific Accell siRNA Delivery

Protocol

This protocol is for Thermo Scientific Accell siRNA Delivery.

PLEASE READ: Thermo Scientific Accell™ siRNA is specially modified for use without a transfection reagent and works at a higher concentration than conventional siRNA with minimal disruption of the expression profile.

The following is a general protocol for use with Accell siRNA in mammalian cells. This protocol was developed for use with adherent cells in a 96-well plate format; however, it may be adopted for nearly any cell type and culture plate or well format.

Each experiment should include the following samples in triplicate:

1. Untreated cells (cells growing in Accell Delivery Media or any other low- or no-serum media. See FAQ section below).
2. Accell positive control siRNA (targeting an endogenous or reporter gene).
3. Negative control siRNA (Accell Non-targeting siRNA control).
4. The desired test siRNA targeting a gene of interest.

All calculations are shown for triplicate samples in duplicate in a 96-well format (three wells per sample on each of two plates). To account for loss during pipetting, all volumes are multiplied by a factor of 1.25.

Delivery Protocol for Adherent Cells

Perform all steps of protocol in a laminar flow cell culture hood using sterile techniques.

Optimal cell densities will vary with growth characteristics of specific cell types. It is recommended to assess the growth rate of your cells in Accell Delivery Media prior to carrying out Accell siRNA silencing experiments.

1. Trypsinize and count cells.
2. Dilute cells in growth media to a plating density of 15-75% confluency (depending upon growth rate of cells and requirements of end point assay).
3. Plate 100 µL of cells at the appropriate density into each well of a 96-well plate.
4. Incubate cells at 37 °C with 5% CO_2 overnight.
5. Dilute 5x siRNA Buffer (Cat. #B-002000-UB-100) to 1x siRNA Buffer by mixing four volumes of sterile RNase-free water (Cat. #B-002000-WB-100) with one volume of 5x siRNA Buffer.
6. Prepare a 100 µM siRNA solution in 1x siRNA Buffer or another appropriate RNase-free buffered solution. For a detailed resuspension protocol and tips on accurate spectrophotometry readings see the siRNA Resuspension Protocol on thermoscientificbio.com.
   a. Resuspend siRNA using the appropriate volume of 1x siRNA Buffer or RNase-free solution.
   b. Pipette solution up and down 3-5 times while avoiding introduction of bubbles.
   c. Place the solution on an orbital mixer/shaker for 70-90 minutes at 37 °C (recommended) or at room temperature.
   d. Briefly centrifuge to collect solution to bottom of the tube/wells.

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7. In separate tubes (or wells of a deep-well plate), mix 7.5 µL of the 100 µM siRNA with 750 µL Accell Delivery Media (Cat. #B-005000). This is the delivery mix and can be used immediately. The final concentration will be 1 µM Accell siRNA per well in a 96-well plate (also see "Protocol variation 1" for serum-sensitive cells).
8. Remove the growth media from the cells and add 100 µL of the appropriate delivery mix (Accell siRNA and delivery media) to each well.
9. Incubate cells at 37 ºC with 5% CO_2 for 72 hours. Longer incubation (96 hours) may be required for protein knockdown.
10. Assess mRNA knockdown. Longer incubation (96+ hours) may be required for protein knockdown. See "Protocol variation 2" below for protein knockdown detection or assays requiring a longer silencing time point.

Delivery Protocol for Suspension Cells

Perform all steps of protocol in a laminar flow cell culture hood using sterile techniques.

The following protocol is recommended for delivery to cells that grow in suspension in a 96-well format. Optimal cell densities will vary with growth characteristics of specific cell types. It is recommended to assess the growth rate of your cells in Accell Delivery Media prior to carrying out Accell siRNA silencing experiments.

1. Dilute 5x siRNA Buffer (Cat. #B-002000-UB-100) to 1x siRNA Buffer by mixing four volumes of sterile RNase-free water with one volume of 5x siRNA Buffer.
2. Prepare a 100 µM siRNA solution in 1x siRNA buffer or another appropriate RNase-free buffered solution. For a detailed resuspension protocol and tips on accurate spectrophotometry readings see the siRNA Resuspension Protocol on thermoscientific.com/onebio.
   a. Resuspend siRNA to using the appropriate volume of 1x siRNA Buffer or RNase-free solution.
   b. Pipette solution up and down 3-5 times while avoiding introduction of bubbles.
   c. Place the solution on an orbital mixer/shaker for 70-90 minutes at 37 °C (recommended) or at room temperature.
   d. Briefly centrifuge to collect solution to bottom of the tube/wells.
3. In separate tubes (or wells of a deep-well plate), 7.5 µL of the 100 µM siRNA.
4. Following general cell culture protocols, count the number of suspension cells in a flask.
5. Spin down the cells and remove the growth media.
   a. Preparations from whole blood may required 2-3 rinses with 1x PBS or Accell delivery media to remove remaining plasma factors or remnants of the separation protocol (such as Ficoll®) that may interfere with Accell application.
6. Resuspend your cells in the appropriate volume of Accell siRNA Delivery Media (Cat. #B-005000). This will depend on the final number of cells desired per well in a 96-well plate (also see "Protocol variation 1" below for serum-sensitive cells).
7. Add 750 µL of the cells plus delivery media mix to the siRNA in the tube or deep-well.
8. Mix gently and add 100 µL of the delivery mix plus cells to each well in a 96-well plate.
9. Incubate cells at 37 ºC with 5% CO_2 for 72 hours.
10. Assess mRNA knockdown. Longer incubation (96+ hours) may be required for protein knockdown. See "Protocol variation 2" below for protein knockdown detection or assays requiring a longer silencing time point.

Protocol variation 1:

If indicated by cell-or assay-dependent requirements, supplement the Accell delivery mix with up to 2.5% serum or additional serum-free supplements (such as Growth factors). Growth media may also be added back as early as 48 hours into the Accell application.

Protocol variation 2:

If indicated by assay-dependent requirements (such as knockdown detection of a long-lived protein), change back to growth media and incubate at 37 ºC with 5% CO_2 for an additional 24+ hours following the standard 72 hour Accell incubation prior to assessing mRNA or protein knockdown.

If cells are tolerant to the Accell application conditions, simply use a 96 hr (or greater) incubation prior to knockdown assessment without an intermediate media change.

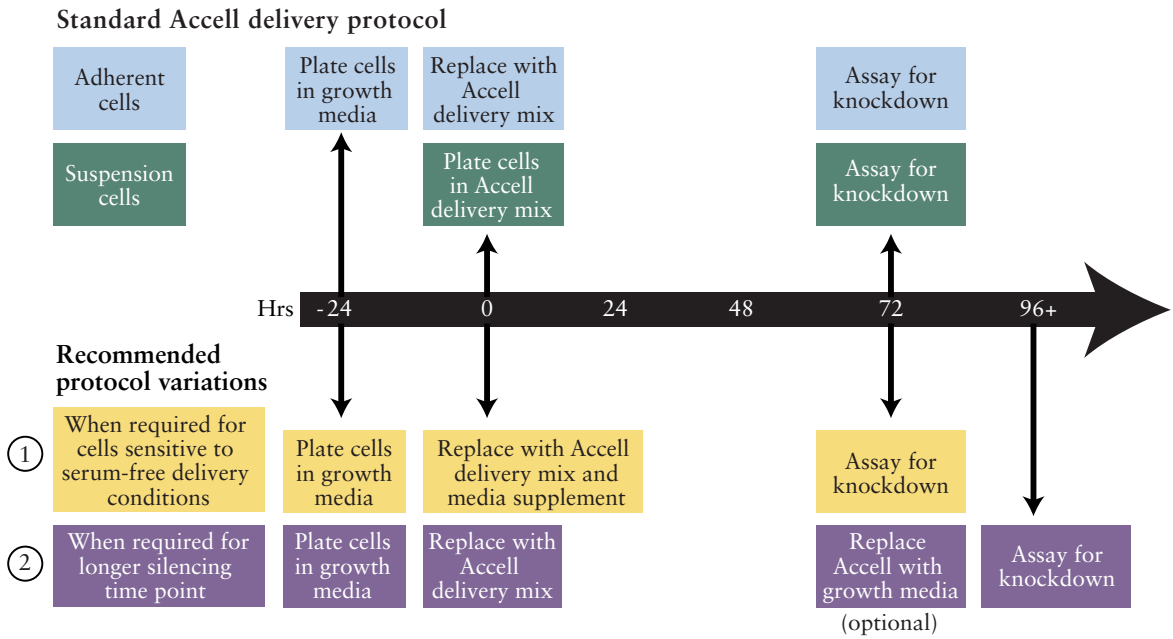

Plate coatings, media, and supplements assessed during Accell siRNA delivery

Plate Coatings	Cell line/type tested	Comments
Gelatin	**Mouse ES-D3** (embryonic stem cells)	No interference with knockdown
Poly-L-lysine	**HeLa, SH-SY5Y and MCF7**	No interference with knockdown
0.001% Fibronectin	**HeLa**	Some interference with knockdown
0.001% Fibronectin	**HUASMC**	Substantially reduces knockdown
0.001% Fibronectin	**HUVEC**	No interference with knockdown
Media/Media Supplements		
Thermo Scientific Hyclone Cell Boost 1	**HeLa, SH-SY5Y**	No interference with knockdown
Hyclone Cell Boost 2™	**HeLa, SH-SY5Y**	No interference with knockdown
Hyclone Cell Boost 3	**HeLa, SH-SY5Y**	No interference with knockdown
Hyclone Cell Boost 4	**HeLa, SH-SY5Y**	No interference with knockdown
HUVEC complete media (contains 2% serum)	**HUVEC**	No interference with knockdown
Astrocyte basal media (ABM; contains no serum)	**NHA** (normal human astrocytes)	No interference with knockdown
Serum* up to 2.5%	**MCF7, SH-SY5Y**	NIH/3T3 Minimal interference with knockdown. Improves cell viability.
Neurobasal™ media (Invitrogen) **no serum, supplemented with Gibco® B27**	**Primary rat cortical neurons**	Data provided by customer; no intereference with knockdown, improves cell viability
Gibco® B27 neuronal supplement	**Mouse brain slices**	Data provided by customer; no intereference with knockdown, improves cell viability

**We recommend minimizing serum concentration whenever possible.*

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