PROTOCOL

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INSPIRED CELL SOLUTIONS

Dharmacon™ Accell™ siRNA delivery

PLEASE READ

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 adapted 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 medium. 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 medium 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-003000-WB-100) with one volume of 5x siRNA Buffer.
6. Add 750 µL of the cells plus delivery media mix to 7.5 µL of the 100 µM siRNA in the tube or deep-well. The final concentration will be 1 µM Accell siRNA per well in a 96-well plate..
   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.
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 medium 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 °C 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.

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
   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. Following general cell culture protocols, count the number of suspension cells in a flask.
4. Spin down the cells and remove the growth medium.
   a. Preparations from whole blood may require 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.
5. 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).
6. Add 750 µL of the cells plus delivery media mix to 7.5 µL of the 100 µM siRNA in the tube or deep-well. The final concentration will be 1 µM Accell siRNA per well in a 96-well plate.
7. Mix gently and add 100 µL of the delivery mix plus cells to each well in a 96-well plate.
8. Incubate cells at 37 °C with 5% CO_2 for 72 hours.
9. 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 medium 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 medium and incubate at 37 °C with 5% CO_2 for an additional 24+ hours following the standard 72 hours Accell incubation prior to assessing mRNA or protein knockdown. If cells are tolerant to the Accell application conditions, simply use a 96 hours (or greater) incubation prior to knockdown assessment without an intermediate medium change.

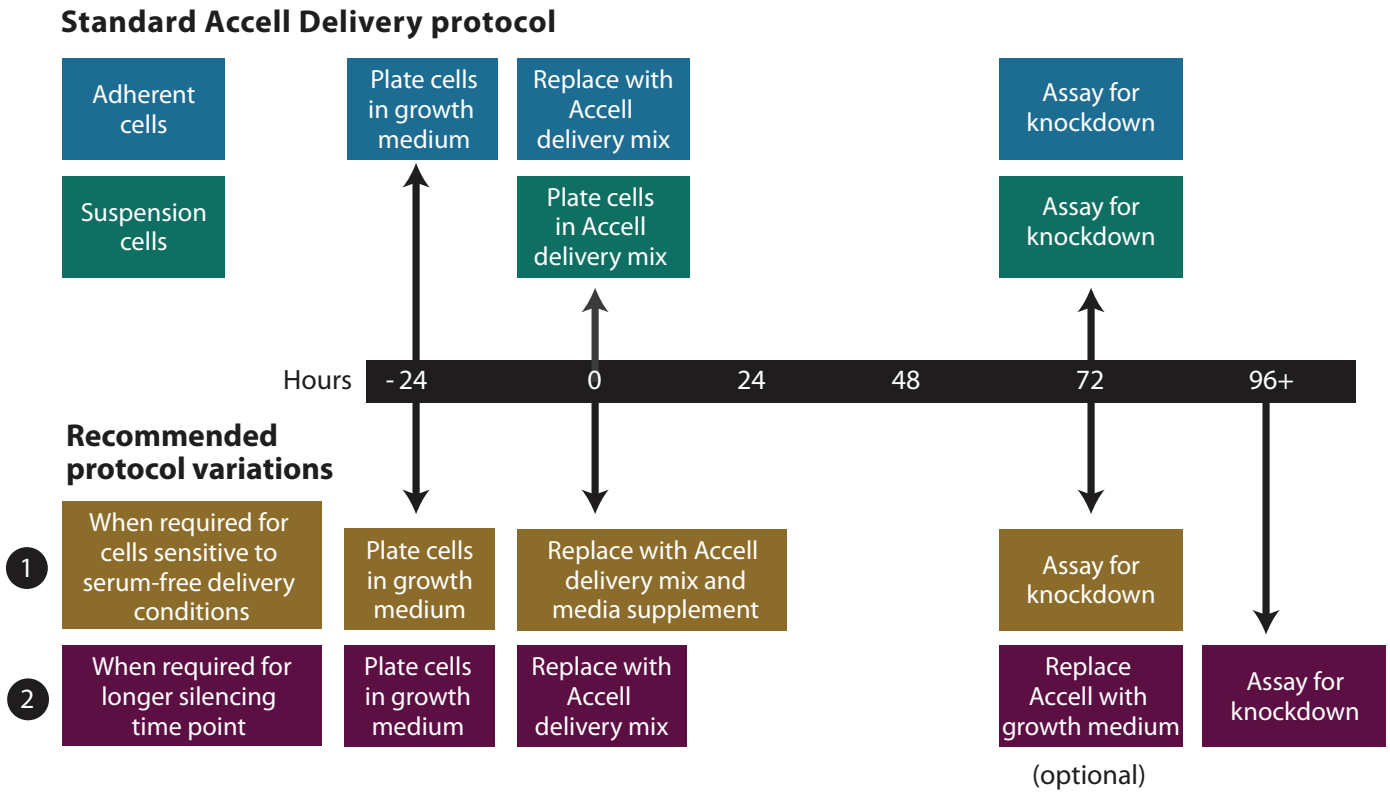

Plate coatings, medium, and supplements assessed with Accell siRNA Delivery Media.

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	**Cell line/type tested**	**Comments**
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 medium (contains 2% serum)	HUVEC	No interference with knockdown
Astrocyte basal medium (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 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.*

Each Accell siRNA product is covered by one or more of the following patents

US8252755, US8501706, US8188060 and US8415466.

If you have any questions, contact

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