

Thermo Scientific Dharmacon

Accell siRNA Delivery Protocol

PLEASE READ: Dharmacon® 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.

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 μ L of cells at the appropriate density into each well of a 96-well plate.
4. Incubate cells at 37°C with 5% CO₂ overnight.
5. 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.
6. Prepare a 100 μ M siRNA solution in 1X siRNA buffer or another appropriate RNase-free buffered solution.
 - a. Use standard resuspension methods. For a detailed resuspension protocol see the Basic siRNA Resuspension protocol on www.thermo.com/dharmacon
7. In separate tubes (or wells of a deep-well plate), mix 7.5 μ L of the 100 μ M siRNA with 750 μ L Accell delivery media (Cat.# B-005000). This is the delivery mix and can be used immediately. The final concentration will be 1 μ M Accell siRNA per well in a 96-well plate (also see “protocol variation 1” below for serum-sensitive cells).
8. Remove the growth media from the cells and add 100 μ L of the appropriate delivery mix (Accell siRNA and delivery media) to each well.
9. Incubate cells at 37°C with 5% CO₂ for 72 hours.
10. Assess mRNA or protein knockdown (also see “protocol variation 2” below for knockdown detection 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 μ M siRNA solution in 1X siRNA buffer or another appropriate RNase-free buffered solution.
 - a. Use standard resuspension methods. For a detailed resuspension protocol see the Basic siRNA Resuspension protocol on www.thermo.com/dharmacon.
3. In separate tubes (or wells of a deep-well plate), add 7.5 μ L of the 100 μ M 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 (e.g. Ficoll®) that may interfere with Accell application.
- 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).
 - Add 750 µL of the cells plus delivery media mix to the siRNA in the tube or deep-well.
 - Mix gently and add 100 µL of the delivery mix plus cells to each well in a 96-well plate.
 - Incubate cells at 37°C with 5% CO₂ for 72 hours.
 - Assess mRNA or protein knockdown (also see “Protocol variation 2” below for knockdown detection requiring a longer silencing time point)

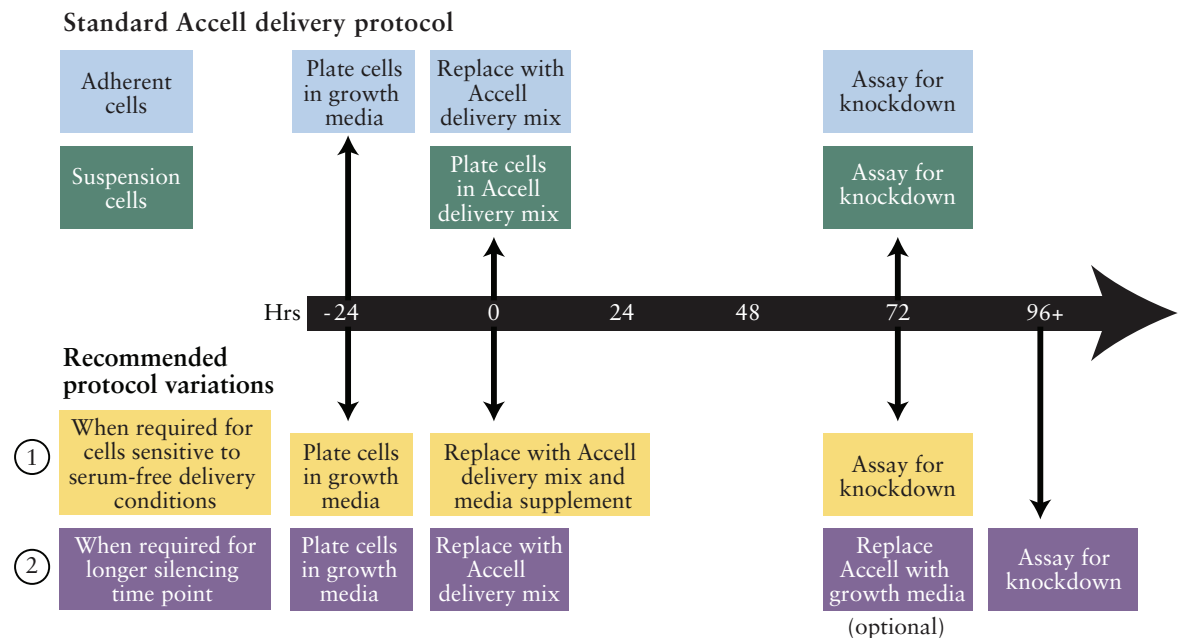
Protocol variation 1:

If indicated by cell or assay-dependent requirements, supplement the Accell delivery mix with 1-3% serum or additional serum-free supplements (e.g. Growth factors).

Protocol variation 2:

If indicated by assay-dependent requirements (e.g. knockdown detection in a long-lived protein), Change back to growth media and incubate at 37°C with 5% CO₂ for an additional 24+ hrs following the standard 72 hr 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.



Frequently Asked Questions

For additional Frequently Asked Questions (FAQs), please visit www.thermo.com/dharmaconFAQs

Question	Solution
Can I substitute any culture media for the Accell delivery media?	The use of media other than the Accell delivery media as an alternative is not recommended and may affect efficiency of Accell siRNA delivery. However, for some cell lines that have specific media requirements, we have found that delivery could be performed in other serum-free or low serum media (1-3% serum) or in the presence of media supplements (see table below) with slight or no change in efficacy.
My cells require certain supplements for optimal growth and assay conditions. May I add these supplements to the Accell delivery mix?	Most serum-free cell culture media supplements may be added to Accell delivery media without detrimental effects on silencing, but will need to be determined empirically in each case. For example, customers have reported excellent viability and target silencing results with cultured brain slices when supplementing the Accell delivery media with Gibco® B-27 serum-free supplement. Please refer to the table below for supplements and media that have been assessed with Accell siRNA.
My cells require serum in the growth media, can I supplement the Accell delivery media with additional serum?	Serum may be added to the delivery media to 1-3%. Our testing shows improved viability with minimal interference with knockdown.

<i>Question</i>	<i>Solution</i>
Do I need to optimize for cell density?	This protocol will work over a wide range of cell densities and plate formats. It is important to use optimal cell density for your cell type and end point assay. If optimization is desired, assess knockdown over a range of cell densities.
How soon can the delivery mix be replaced with growth medium?	72 hrs is the optimal duration for Accell application. We recommend that cells are cultured in the delivery mix for at least 48 hrs before changing media but that a full 72 hrs should elapse prior to assay for silencing.
Can I use less than 1 μ M Accell siRNA to see efficacy?	Due to the nature of passive delivery, we recommend 1 μ M as a starting concentration. Because there is no transfection reagent used, there is minimal toxicity or off-targets associated with this concentration. Titration to 500 nM or 200 nM has been successful with many gene targets.
What is the stability of the Accell delivery mix?	The delivery mix (Accell siRNA diluted in Accell delivery media) is stable for at least 90 days when stored at 4°C.

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

<i>Plate Coatings</i>	<i>Cell line/type tested</i>	<i>Comments</i>
Gelatin	Mouse ESD3 (embryonic stem cells)	No interference with knockdown
Poly-L-lysine	HeLa, SH-SY5Y and MCF-7	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
<i>Media/Media Supplements</i>		
Thermo Scientific Hyclone Cell Boost 1™	HeLa, SH-SY5Y	No interference with knockdown
Thermo Scientific Hyclone Cell Boost 2™	HeLa, SH-SY5Y	No interference with knockdown
Thermo Scientific Hyclone Cell Boost 3™	HeLa, SH-SY5Y	No interference with knockdown
Thermo Scientific 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 3%	MCF-7, SH-SY5Y, 3T3 NIH	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 interference with knockdown, improves cell viability
Gibco® B27 neuronal supplement	Mouse brain slices	Data provided by customer; no interference with knockdown, improves cell viability

*We recommend minimizing serum concentration whenever possible.

Contact Information

For technical questions regarding the use of siRNA reagents, please contact Dharmacon Products Technical Support at:

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