Technical Manual

Thermo Scientific Open Biosystems Arrest-In Transfection Reagent

Catalog #: ATR1740, ATR1741, ATR1742, ATR1743

Product Description

Open Biosystems Arrest-In™ Transfection Reagent is a proprietary polymeric formulation, developed and optimized for highly efficient delivery of shRNA plasmid DNA into the nucleus of cultured eukaryotic cells. It is well known that polymers, but not cationic lipids, protect DNA in the cytoplasm and promote entry into the nucleus of transfected cells (Pollard *et al.* 1998). Arrest-In Transfection Reagent also provides an enhanced uptake efficiency of the shRNA plasmid DNA into cells. Once in the cells Arrest-In promotes the entry of the shRNA containing plasmid into the nucleus where it is transcribed into a hairpin, enters the cytoplasm and is processed by the endogenous RNAi machinery into functional siRNAs.

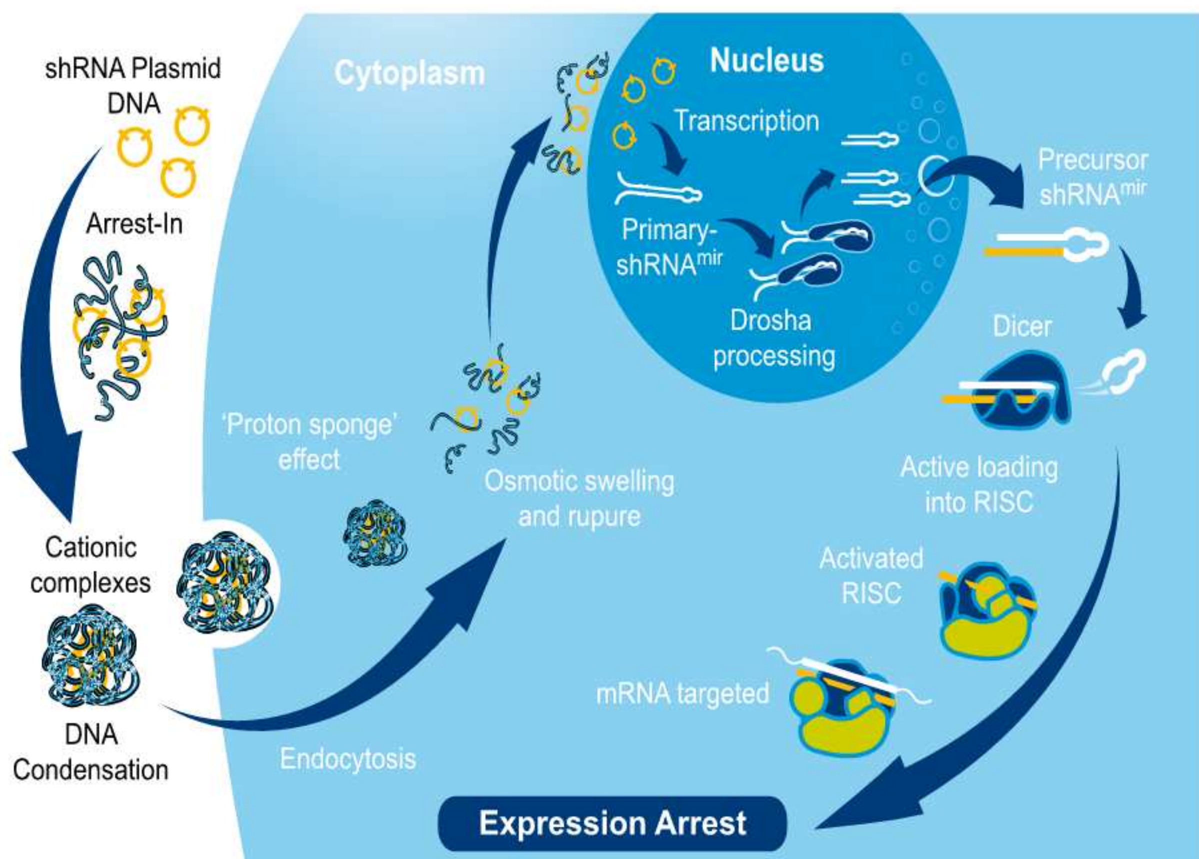

Figure 1. Schematic of plasmid-based shRNAmir expression and processing. shRNAmir plasmid forms complexes with Arrest-In. This protects the plasmid DNA as it enters the cell and facilitates it uptake into the nucleus. The shRNAmir are transcribed from the plasmid and exported to the cytoplasm. They are then processed by the endogenous microRNA biosynthetic pathway resulting in efficient knockdown.

Arrest-In is easy to use, robust, and exhibits very low toxicity. One milliliter is sufficient for approximately 100 transfections on 35 mm tissue culture dishes using 2 µg of DNA.

Shipping And Storage

Arrest-In is supplied in sterile filtered water at a concentration of 1 mg/ml. Arrest-In is available in 0.5 ml, 1 ml, 5x1 ml and 10x1 ml volumes.

Arrest-In is shipped on wet ice. Store at 4°C for up to 12 months.

Quality Control

Each lot of Arrest-In is functionally tested by transfection of HEK293T cells with 500 ng reporter plasmid. Following transfection, the cells are assayed at 48 hours and typically >70% of the cells will be positive for reporter activity. Each lot is tested by NMR and elemental analysis.

Protocol For Delivery Of shRNA Plasmid DNA

The protocol below is optimized for transfection of the shRNA plasmid DNA into HEK293T cells in a 24-well plate using serum-free media. If a different culture dish is used, adjust the number of cells, volumes and reagent quantities in proportion to the change in surface area (see Table 1).

Table 1. Suggested amounts of DNA, medium and Arrest-In reagent for transfection of shRNA plasmid DNA into adherent cells.

Tissue Culture Dish	Surface Area Per Plate or Well (cm^2)	Total Serum-Free Media Volume Per Well (ml)	Plasmid DNA (µg)*	Arrest-In (µg)**
60 mm	20.0	2.0	4.0	21.0
35 mm	8.0	1.0	2.0	10.0
6-well	9.4	1.0	2.0	10.0
12-well	3.8	0.5	1.0	5.0
24-well	1.9	0.25	0.5	2.5
96-well	0.3	0.1	0.1 - 0.2	0.5 - 1.0

*Recommended starting amount of DNA. May need to be optimized for the highest efficiency.
**Recommended starting amount of Arrest-In reagent. See Transfection Optimization.

It is preferable that transfections be carried out in medium that is serum-free and antibiotic free. A reduction in transfection efficiency occurs in the presence of serum, however it is possible to carry out successful transfections with serum present (see Transfection Optimization).

Warm Arrest-In to ambient temperature (approximately 20 minutes at room temperature) prior to use. Always mix well by vortex or inversion prior to use.

Maintain sterile working conditions with the DNA and Arrest-In mixtures as they will be added to the cells.

1. The day before transfection (day 0), plate the cells at a density of 5×10^4 cells per well of a 24-well plate.
   Full medium (i.e. with serum and antibiotics) will be used at this stage.
2. On the day of transfection, form the DNA/Arrest-In transfection complexes.
   The principle is to prepare the shRNA plasmid DNA and transfection reagent dilutions in an equal amount of serum-free medium in two separate tubes. These two mixtures (i.e. the DNA and the Arrest-In) will be added to each other and incubated for 20 minutes prior to addition to the cells. This enables the DNA/Arrest-In complexes to form.
   a. For each well to be transfected, dilute 500 ng shRNA plasmid DNA into 50 µl (total volume) of serum-free medium in a microfuge tube.
   b. For each well to be transfected, dilute 2.5 µg (2.5 µl) of Arrest-In into 50 µl (total volume) serum-free medium into a separate microfuge tube.
   c. Add the diluted DNA (step a) to the diluted Arrest-In reagent (step b), mix rapidly then incubate for 20 minutes at room temperature.
      This will give a 1:5 DNA:Arrest-In ratio which is recommended for optimal transfection into HEK293T cells. Your total volume will be 100 µl at this stage.
   d. Set up all desired experiments and controls in a similar fashion as outlined in Table 2. It is also advisable to set up an Arrest-In only control.

Table 2. Quantities of DNA for transfection experiments.

Type of Transfection Experiment	shRNA Plasmid DNA (ng)	Reporter* (ng)	Carrier DNA** (ng)	Serum-Free Medium (Final Volume in µl)
shRNA plasmid DNA	500 – hairpin to gene of interest	0	0	50
Transfection efficiency	0	500	0	50
Knockdown efficiency of reporter	450-500 – hairpin to reporter	50	0	50
Control for knockdown efficiency	0	50	450 - 500	50
Non-silencing control	500 – scramble hairpin	0	0	50

*It is not necessary to transfect a reporter into cells if you are using a construct which already has a reporter for convenient estimation of transfection efficiency. Recommended reporters for other vectors include GFP, luciferase and/or β-gal (X-gal staining and/or ONPG assays).
**Carrier DNA is required to increase the total DNA quantity for the formation of adequate DNA/Arrest-In complexes. Recommended carriers are pUC19 or pBluescript plasmids.

3. Aspirate the growth medium from the cells. Add an additional 150 µl of serum-free medium to each of the tubes containing transfection complexes and mix gently. Add the 250 µl DNA/Arrest-In complex mixture to the cells and incubate for 3-6 hours in a CO_2 incubator at 37°C.
   Your total volume will be 250 µl at this stage.

4. Following the 3-6 hour incubation, add an equal volume of growth medium (250 µl) containing twice the amount of normal serum to the cells (i.e. to bring the overall concentration of serum to what is typical for your cell line). Alternatively, the transfection medium can be aspirated and replaced with the standard culture medium (see note). Return the cells to the CO_2 incubator at 37°C.
   Note – Arrest-In has displayed low toxicity in the cell lines tested, therefore removal of transfection reagent is not required for many cell lines. In our experience, higher transfection efficiencies have been achieved if the transfection medium is ***not*** *removed. However, if toxicity is a problem, aspirate the transfection mixture after 3-6 hours and replace with fresh growth medium. Additionally, fresh growth medium should be replenished as required for continued cell growth.*

5. After 48-96 hours of incubation, examine the cells microscopically for the presence of reporter expression where applicable as this will be your first indication as to the efficiency of your transfection. Then assay cells for reduction in gene or reporter activity by quantitative/real-time QPCR, western blot or other appropriate functional assay; compare to untreated, reporter alone, non-silencing shRNA or other negative controls.
   Optimal length of incubation from the start of transfection to analysis is dependent on cell type, gene of interest, and the stability of the mRNA and/or protein being analyzed. Quantitative/real-time QPCR generally gives the best indication of expression knock-down. The use of western blots to determine knock-down is very dependent on quantity and quality of the protein, its half-life, and the sensitivity of the antibody and detection systems used.

6. If selecting for stably transfected cells (optional), transfer the cells to medium containing puromycin for selection. It is important to wait at least 48 hours before beginning selection.
   The working concentration of puromycin needed varies between cell lines. We recommend you determine the optimal concentration of puromycin required to kill your host cell line prior to selection for stable shRNA transfectants. Typically, the working concentration ranges from 1-10 µg/ml. You should use the lowest concentration that kills 100% of the cells in 3-5 days from the start of puromycin selection.

Cells Grown In Suspension

Transfection of cells in suspension follows all the above principles and the protocol remains largely the same, except that the DNA/Arrest-In mixture is added to cells (post 20 minute incubation for complex formation) to a total volume of 250 µl serum-free medium or to a total volume of 250 µl of medium with serum (no antibiotics).

Transfection Optimization Using Arrest-In

It is essential to optimize transfection conditions to achieve the highest transfection efficiencies and lowest toxicity with your cells. The most important parameters for optimization are DNA to transfection reagent ratio, DNA concentrations and cell confluency. We recommend that you initially begin with the Arrest-In and DNA amount indicated in Table 1 and extrapolate the number of cells needed for your vessel size from the number of cells used in a well of a 24-well plate as listed in step 1 of the protocol for delivery of plasmid DNA.

How Many Transfections Are Available In Each Volume Size of Arrest-In?

The number of transfections that can be performed depends on the size of the culture dish used and the volume size of Arrest-In purchased. Refer to Table 3 for the approximate number of transfections.

Table 3. Number of transfections depending on culture dish size and volume of Arrest-In purchased.

Tissue Culture Dish	Surface Area Per Well (cm^2)	Arrest-In (1mg/ml) (µg)*	0.5 ml Qty (rxns)**	1.0 ml Qty (rxns)**	5.0 ml Qty (rxns)**	10 ml Qty (rxns)**
60 mm	20.0	21.0	47 - 50	100	500	1000
35 mm	8.0	10.0	100	200	1000	2000
6-well	9.4	10.0	100	200	1000	2000
12-well	3.8	5.0	200	400	2000	4000
24-well	1.9	2.5	400	800	4000	8000
96-well	0.3	0.5 - 1.0	1000	2000	10000	20000

*Recommended starting amounts of Arrest-In reagent as defined in Table 1.

**Approximate number of transfections based on recommended starting amount of Arrest-In. Individual results may vary depending on amounts of Arrest-In used.

What Cell Lines Have Been Successfully Transfected Using Arrest-In?

Refer to Table 4 for a list of cell lines that have been successfully transfected using Arrest-In.

Table 4. Cell lines transfected with Arrest-In.

HEK293	PC12
HEK293T	MO3
Cos	Ava5
Jurkat	MG63
CHO	3T3
HepG2	MC57
Mouse ES	HUVEC
Huh7	RAW
5ySy	NT2

Troubleshooting

If transfection into your cell line is unsuccessful, you may need to consider the following list of factors influencing successful transfection.

a. Concentration and purity of plasmid DNA and nucleic acids: Determine the concentration of your DNA using 260 nm absorbance. Avoid cytotoxic effects by using pure preparations of nucleic acids.

b. Insufficient mixing of transfection reagent or transfection complexes.

c. Transfection in serum-containing or serum-free media: Our studies indicate that Arrest-In/DNA complexes should preferably be formed in the absence of serum. In the cell lines tested we found that the highest transfection efficiencies can be obtained if the cells are exposed to the transfection complexes in serum-free conditions followed by the addition of medium containing twice the amount of normal serum to the complex medium 3-6 hours post transfection (leaving the complexes on the cells). However, the serum-free transfection medium can be replaced with normal growth medium if high toxicity is observed.

d. Presence of antibiotics in transfection medium: The presence of antibiotics can adversely affect the transfection efficiency and lead to increased toxicity levels in some cell types. It is recommended that antibiotics be excluded until transfection has mostly occurred (3-6 hours) and then be added together with the full medium.

e. High protein expression levels: Some proteins when expressed at high levels can be cytotoxic; this effect can also be cell line specific.

f. Cell history, density, and passage number: It is very important to use healthy cells that are regularly passaged and in growth phase. The highest transfection efficiencies are achieved if cells are plated the day before, however, adequate time should be given to allow the cells to recover from the passaging (generally >12 hours). Plate cells at a consistent density to minimize experimental variation. If transfection efficiencies are low or reduction occurs over time, thawing a new batch of cells or using cells with a lower passage number may improve the results.

If Arrest-In seems to be toxic to a particular cell line, try reducing the DNA:Arrest-In ratio to reduce toxicity.

References

Pollard H., Remy J.S., Loussouarn G., Demolombe S.,Behr J.P., Escande D. Polyethylenimine but not cationic lipids promotes transgene delivery to the nucleus in mammalian cells. J Biol Chem. 1998; 273(13):7507-11.

FAQS/Troubleshooting

For answers to questions that are not addressed here, please email technical support at openbiosystems@thermofisher.com with your question, your sales order or purchase order number and the catalog number or clone ID of the construct or collection with which you are having trouble.

Contact Information
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