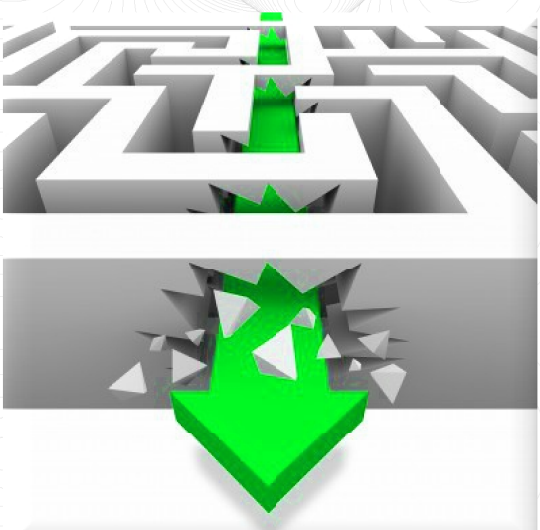
BioCellChallenge

Your Partner in Drug Delivery

GeneCellin™ HTC

Transfection Reagent dedicated to Hard to Transfect Cells



GeneCellin™ HTC
Transfection Reagent Protocol

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Description

GeneCellin™ HTC is a powerful *in vitro* transfection reagent which allows to achieve higher delivery efficiencies of plasmid DNA into Hard to Transfect Cells. This new polymer based reagent is ideal to transfect primary cells, stem cells and many other hard to transfect cell lines. It allows achieving a maximum transgene expression level without the need for sophistical materials.

Content

GeneCellin™ HTC transfection reagent is available in several sizes:

Reference	Size	Number of transfections in a 24-well plate
HTC-100	100 µL	50
HTC-750	750 µL	375
HTC-3750	5 x 750 mL	1875

Storage

GeneCellin™ HTC should be stored at 4°C upon receipt. GeneCellin™ HTC reagent is stable for at least one year at 4°C.

Certificate of Quality

1- Efficiency and non toxicity of GeneCellin™ HTC reagent is guaranteed by testing each batch of reagent with plasmid DNA transfection experiments into Raw264 and rat mesenchymal stem cells.
2- Sterility is controled by thioglycolate assay.
3- GeneCellin™ HTC is certified free of animal origin contaminants.

Parameters influencing transfection efficiency

● ***Nucleic acids purity***

Presence of high level of endotoxins in plasmid DNA preparation could lead to lower transfection efficiencies or cause high cellular toxicity. We recommend the use of high quality endotoxin-free DNA preparation kit.

● ***Cell density***

We recommend that the cells are 60-70 % confluent at the day of transfection.

● ***Presence of serum or antibiotics***

The presence of serum and/or antibiotics in the culture medium do not interfere with GeneCellin™ HTC transfection efficiencies. Cells can be maintained in their regular culture medium during the transfection.

● ***Mycoplasma contamination***

Mycoplasma infection in cell culture results in poor and non-reproducible transfection.

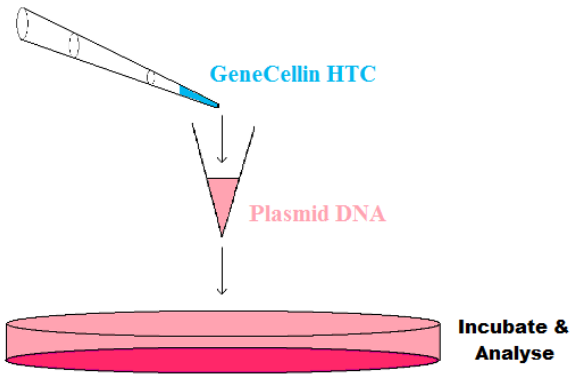
Successfully transfected cells

GeneCellin™ HTC has been successfully and extensively tested in various adherent and suspension cell lines in our laboratories and by our customers testing panel. Please visit our website www.biocellchallenge.com for having an updated list of successfully transfected cells with GeneCellin™ HTC transfection reagent. Do not hesitate to contact us if you need optimized protocols for your cell types.

Primary Cells	Stem Cells	Cell Lines
Mouse macrophages	Mouse mesenchymal	HepG2
Human lung fibroblasts	Rat mesenchymal	HaCat
Mouse dendritic cells	Mouse embryonic	Raw264
Rat embryonic fibroblast	/	MDCK
Mouse lymphoma	/	HCT116
Human neuroblastoma	/	SKNAS
Mouse cardiac myocytes	/	MEF

Transfection protocol

● ***Principle***



● ***Cell culture***

Seed adherent healthy cells the day before transfection according to Table 1 below so they could be 60-70% confluent on the day of transfection.

Split suspension healthy cells the day before transfection according to Table 1 below so they could be in logarithmic growth phase at the time of transfection.

● ***Transfection protocol***

Transfection protocol is provided for a 24 well-plate culture vessel and for adherent cells. See Table 1 to adapt your protocol in other culture formats.

1- Seed 50,000 adherent cells the day before transfection in 1 mL of serum containing culture medium.

2- Dilute 1 µg of DNA in 100 µL of serum free medium (DMEM, RPMI or other growth medium).

3- Add 2 µL of GeneCellin™ HTC to the diluted DNA solution and mix the solution by vortexing during 2-3 seconds.

4- Incubate 15 minutes at room temperature.

5- Add the 100 µL of GeneCellin™ HTC / DNA mixture dropwise onto the cells plated in 1 mL of serum containing culture medium and gently rock the plate to ensure an even distribution of the complexes (do not swirl the plate or the dish).

6- Incubate at 37°C in a CO_2 incubator.

7- Analyse transgene expression 24-48 hours later.

Table 1: Transfection conditions

Tissue culture vessel	Number of adherent (suspension) cells to seed	Volume of culture cell medium (µL)	Amount of DNA* (µg)	Volume of DNA solution (µL)	Volume of GeneCellin™ HTC*(µL)
96 -well	8,000 (16,000)	200	0.2	20	0.4
48 -well	20,000 (40,000)	500	0.5	50	1
24 -well	50,000 (100,000)	1,000	1	100	2
12 -well	100,000 (200,000)	2,000	2	200	4
6 -well 35 mm	250,000 (500,000)	4,000	4	400	8
60 mm T25	600,000 (1,200,000)	6,000	6	600	12
100 mm T75	2,000,000 (4,000,000)	15,000	15	1,000	30

* Amounts of DNA and GeneCellin HTC reagent are given as starting points. Depending on cell and experiment types, optimizations may be required.

Optimizations

● ***Cell confluency***

The number of cells to seed indicated in the Table 1 above may need some optimizations according to cell growth.

Depending on cell types, lower or higher confluency conditions could be preferred. We recommend optimizing cell plating conditions when necessary.

● ***Amounts of DNA and GeneCellin HTC***

Amounts of plasmid DNA and/or GeneCellin™ HTC reagent could need to be optimized for some cell types. In this case, we recommend you to follow the amount ranging of DNA and reagents as mentioned in table 2 below.

Table 2: DNA and GeneCellin™ HTC ranges

Tissue culture vessel	Amount ranging of reagents (µL)	
	Plasmid DNA	GeneCellin™ HTC
96-well	0.1-0.4	0.3-0.6
48-well	0.3-0.8	0.5-1.5
24-well	0.5-1.5	1-3
12-well	1-3	2-6
6-well	2-6	6-10
60 mm / T25	4-8	8-16
100 mm / T75	10-20	25-35

Other transfection procedures

● ***Co-transfection***

Respect the total amount of plasmid DNA according to Table 1 above.

● ***Stable transfection***

Cells should be growth in a selective medium for 15 days. Due to high efficiency of GeneCellin™ HTC, you can replace regular culture medium by the selection medium as soon as 24h post-transfection.

Technical support

Do not hesitate to contact our technical scientific team at technical@biocellchallenge.com if you need further information about GeneCellin™ HTC transfection reagent.

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