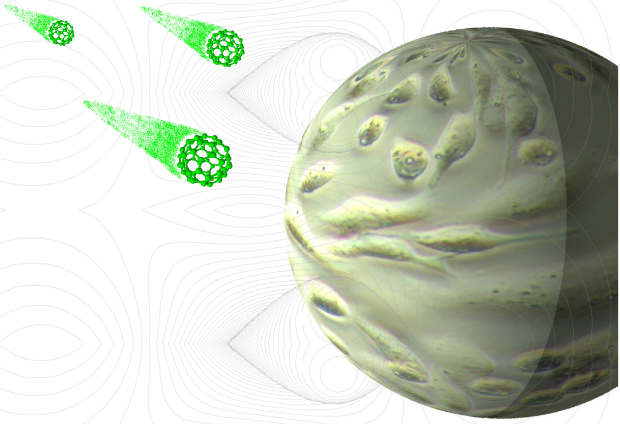
BioCellChallenge

Your Partner in Drug Delivery

GeneCellin™ Transfection Reagent Protocol

From Delivery to Discovery



GeneCellin™ Transfection Reagent Protocol

Table of content

Description

GeneCellin™ is a powerful *in vitro* transfection reagent which allows to achieve higher delivery efficiencies of plasmid DNA into cell lines and primary cells as compared to other lipid and polymer based transfection reagents. This innovative product is composed by a stable formulation of nanoparticules we have named **"nanoporters"**. These allow achieving very good and reproducible transgene expression levels without any cytotoxic effect even with sensitive cells.

Content

GeneCellin™ transfection reagent is available in several sizes:

Reference	Size	Number of transfections in a 24-well plate
GC50 - sample	50 µL	25
GC500	500 µL	250
GC1000	1 mL	500
GC5000	5 x 1 mL	2500

Storage

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

Certificate of quality

1- Efficacy and non toxicity of GeneCellin™ reagent is guaranted by testing each batch of reagent with plasmid DNA transfection experiments into HeLa cell lines.
2- Sterility is controled by thioglycolate assay.
3- GeneCellin™ 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 antibiotics in the culture medium do not interfere with GeneCellin™ 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.

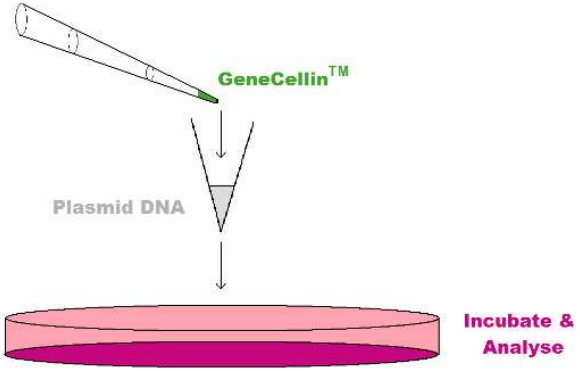
Successfully transfected cells

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

Cell Types	Transfection efficiency %	Cell Types	Transfection efficiency %
A549	60	HEK293	95
B50	60	HeLa	95
B65	35	HepG2	55
BHK21	65	L929	60
Calu-1	70	MCF-7	30
CHO	90	MDCK	85
Colo	20	NIH3T3	95
Cos-7	90	Raw 264	60
HCT-116	75	Vero	50

Transfection protocol

● ***Principle***



● ***Cell culture***

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

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

● ***Transfection***

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

1- Dilute 0.5 µg of DNA in 100 µL of serum free medium (DMEM, RPMI or other growth medium).

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

3- Incubate 15 minutes at room temperature.

4- Add the 100 µL of GeneCellin™ / DNA mixture drop-wise onto the cells plated in 500 µL 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).

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

6- 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™ (µL)
384 well	3,000 (6,000)	50	0.05	10	0.2
96 well	8,000 (16,000)	100	0.15	20	0.5
48 well	20,000 (40,000)	250	0.25	50	1
24 well	50,000 (100,000)	500	0.5	100	2
12 well	100,000 (200,000)	1,000	1	100	4
6 well 35 mm	250,000 (500,000)	2,000	2	200	8
60 mm T25	600,000 (1,200,000)	5,000	4	200	12
100 mm T75	2,000,000 (4,000,000)	10,000	10	500	30

Optimization

● ***Cell confluency***

The number of cells to seed indicated in the Table 1 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.

● ***Amount of plasmid DNA***

Higher or lower amounts of plasmid DNA can be used for hard-to-transfect cells or sensitive cells respectively. In this case, we recommend you trying to use twice more or twice less DNA keeping unchanged the quantities of GeneCellin™ according to Table 1.

Other transfection procedures

● ***Co-transfection***

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

● ***Stable transfection***

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

● ***Reverse transfection***

You can perform a reverse transfection as a one day, time-saving procedure avoiding cell plating before transfection. Proceed as described in the transfection protocol above. Evenly distribute the GeneCellin™ / DNA mixture to the bottom of the culture vessel. Gently layer cells on top of the transfection mixture. We recommend to seed double number of cells compared to regular transfection conditions.

Technical support

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

Product Use Limitation

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor it is suitable for administration to human or animals. Please refer to www.biocellchallenge.com for Material Safety Data Sheet of the product.

The purchase of this product includes a non-transferable licence to use it for the purchaser's internal research only. All other commercial uses of this product require a separate license from BioCellChallenge SAS.