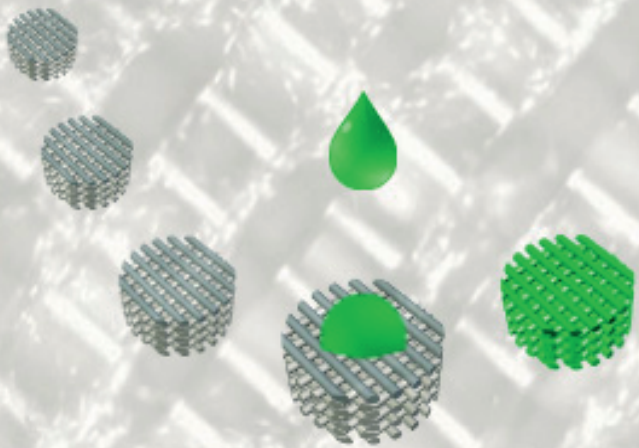
BCC
BioCellChallenge

3D CELL TRANSFECTION KIT

Add a New Dimension to Your Transfection



3D Biotek

3D Cell Transfection Kit - Protocol

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Description

The 3D Cell Transfection Kit is the first and unique *in vitro* transfection technology which allows researchers to achieve a high delivery of plasmid DNA into 3D cultured cells. This innovative product includes a sterile plate containing **3D Cell Culture Scaffolds** (3D Biotek, LLC) and a specially designed **3D Transfection Reagent** (BioCellChallenge, SAS). With this kit, researchers can now perform extended transgene expression time-courses in a physiological-like cell growth environment.

3D Cell Transfection Kit is available in several sizes:

Reference	Scaffold Number	Scaffold Size	3D Transfection Reagent (µL)
3D-DNA96	24	96-well	100
3D-DNA24	12	24-well	200

Scaffold-containing plates are not tissue culture treated. They are specifically adapted for 3D cell culture and should not be used for 2D cell culture.

Storage

The plate containing the 3D Cell Culture Scaffolds can be stored at room temperature. 3D Transfection Reagent is stable for at least 1 year at 4°C.

Certificate of Quality

1- The 3D Cell Transfection Kit's transfection efficiency is guaranteed by testing each batch of 3D Transfection Reagent with plasmid DNA transfection experiments in 3D NIH3T3 cultures.

2- Sterility is controlled by thioglycolate assay.

3- 3D Cell Transfection Kit is certified free of animal origin contaminants.

Parameters Influencing 3D Cell Transfection Efficiency

● ***Cell growth***

We recommend that cells which are seeded onto 3D cell culture scaffolds come from a 60-70% confluent 2D cell culture at the day of transfection.

● ***Cell seeding***

When seeding cells onto 3D Cell Culture Scaffolds, ensure that the cell suspension is pipetted directly onto the top center surface of the scaffolds. If the cell suspension contacts the sides of tissue culture wells, it can significantly lower 3D Cell Transfection efficiencies.

● ***Nucleic acids purity***

The presence of a high level of endotoxins in plasmid DNA preparation could lead to lower 3D transfection efficiencies or cause high cellular toxicity. We recommend the use of a high quality endotoxin-free DNA preparation kit.

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

The presence of serum and antibiotics in the culture medium does not interfere with 3D Cell Transfection efficiencies.

● ***Mycoplasma contamination***

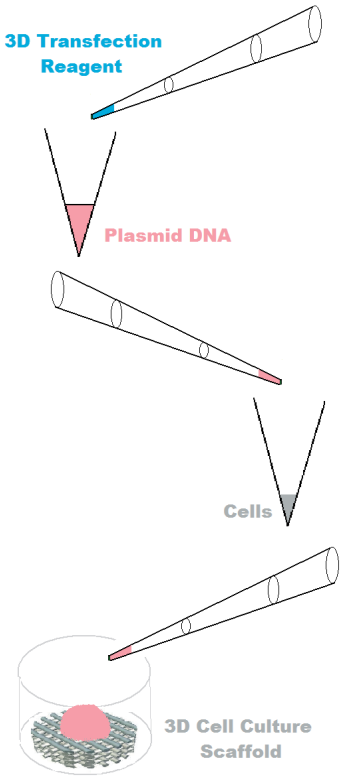
Mycoplasma infection of cell culture results in poor and non-reproducible 3D transfection experiments.

Successfully 3D Transfected Cells

The 3D Cell Transfection Kit has been successfully tested in various adherent cell lines. Please visit our websites at www.biocellchallenge.com or www.3dbiotek.com for an updated list of successfully 3D transfected cells.

3D Transfection Protocol

- ***Principle***



● ***3D Cell Transfection***

A 3D Cell Transfection protocol is provided for a 96-well scaffold. See Table 1 below to adapt your protocol to the other 3D scaffold size.

1- Dilute 0.5 µg of plasmid DNA in 17 µL of serum-free medium (DMEM, RPMI or other growth medium).

2- Add 3 µL of 3D Transfection Reagent to the diluted DNA solution and mix the solution by pipetting.

3- Incubate 15 minutes at room temperature.

4- During this incubation time, spin down 200,000 healthy cells for 5 min at 1,000 rpm in a microtube and discard supernatant.

5- Add the 20 µL of 3D Transfection Reagent / DNA mixture onto the pellet of cells, mix gently by pipetting and slowly pipette this 20 µL suspension cell onto the top center surface of the 3D cell culture scaffold.

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

7- After 3 hours of incubation, slowly add 180 µL of complete medium (containing serum) and return the plate to the CO_2 incubator. Ensure that the scaffold is sitting at the bottom of the well. If not, gently push it down with a pipette tip.

8- After 24 hours, carefully aspirate the medium around the scaffold and then flip and transfer it into a new well with

sterile forceps. Slowly add fresh medium into the well and make sure that the scaffold is sitting at the bottom of the well.

9- Monitor the 3D cell transfection under an inverted light microscope. Analyse the transgene expression or continue the transgene expression for later analyses, according to your experimental protocol.

Any detachment method that you are currently using with 2D cultured cells can be used to detach and analyse cells cultured on our 3D Cell Culture Scaffolds.

● ***3D Cell Detachment***

Rinse twice with PBS, incubate at 37°C with enzyme for 10-30 minutes, add complete medium and flush out the cells from the scaffold by pipetting.

As cells cultured on 3D Cell Culture Scaffolds secrete a significant extracellular matrix, similar to cells in vivo, a longer enzyme incubation period may be necessary for certain cell types.

Table 1: 3D Transfection Conditions

Reference	Number of Cells	Serum-Free Medium (µL)	DNA (µg)	3D Transfection Reagent (µL)	Culture Medium (µL)
3D-DNA96	2×10^5	17	0.5	3	180
3D-DNA24	1×10^6	85	2.5	15	400

Optimization

- ***Cell density***

The number of cells to seed, as indicated in Table 1, may require further optimization as a result of 3D cell growth, cell morphology, and type of experiment planned.

Technical Support

Do not hesitate to contact our technical scientific support teams at technical@biocellchallenge.com or info@3DBiotek.com if you need further information about the 3D Cell Transfection Kit.

Product Use Limitation

This product is developed, designed and sold exclusively for research purposes and is intended for *in vitro* use only. The product was not tested for use in diagnostics or for drug development. It is not suitable for administration to humans or animals. Please refer to www.biocellchallenge.com and www.3DBiotek.com for the product's Material Safety Data Sheet.

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 and 3D Biotek, LLC.

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