

3D-Fect™ transfection reagent - Results

OZ Biosciences is delighted to announce the launching of a new 3D transfection reagent, **3D-Fect™**. 3D-Fect™ is the newest reagent specifically designed and developed for transfection of cells cultured in 3D scaffolds (sponges, matrices, inserts...). This formulation is based on a novel technology that allows adding a third dimension to cell cultures.

Main **3D-Fect™** features are:

1. Highly efficient
2. Ideal for any 3D scaffolds (sponges, matrices, insert)
3. Completely biodegradable
4. Universal (primary cells and cell lines)
5. Multipurpose (various types of nucleic acid)
6. Simple, ready-to-use & rapid
7. Serum compatible
8. Appropriate for multiple applications
9. Long term transgene expression

Nucleic acid types

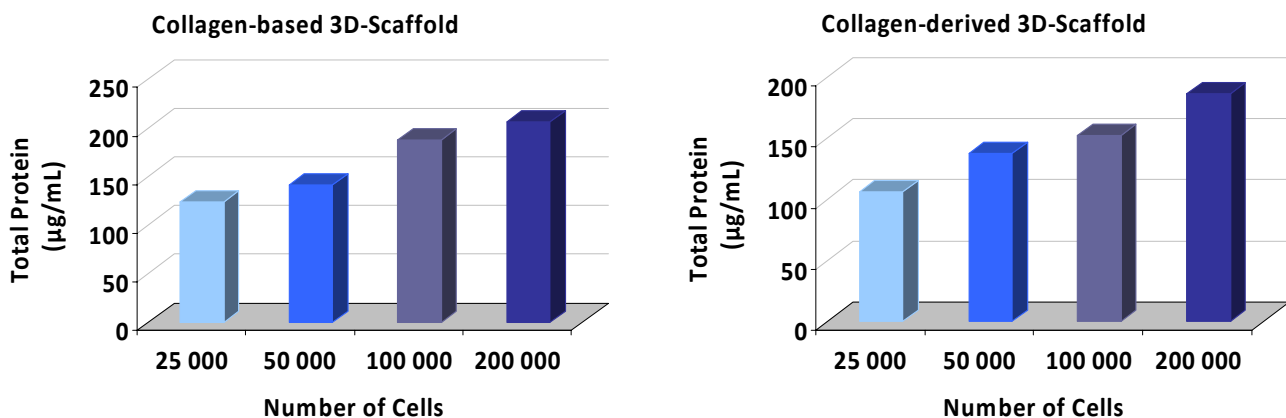
3D-Fect™ transfection reagent is suitable for all type of nucleic acids including: plasmid DNA, siRNA, oligonucleotides, linearized DNA, double stranded RNA, mRNA, shRNA.

Cell types – 3D Scaffolds

3D-Fect™ is suitable for 3D scaffold types (Table 1) and numerous cells (see Table 2). If a particular cell type or scaffold type is not listed, this does not imply that **3D-Fect™** is not going to work. OZ Biosciences is maintaining an updated list of cells and scaffolds successfully tested that is available on the website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

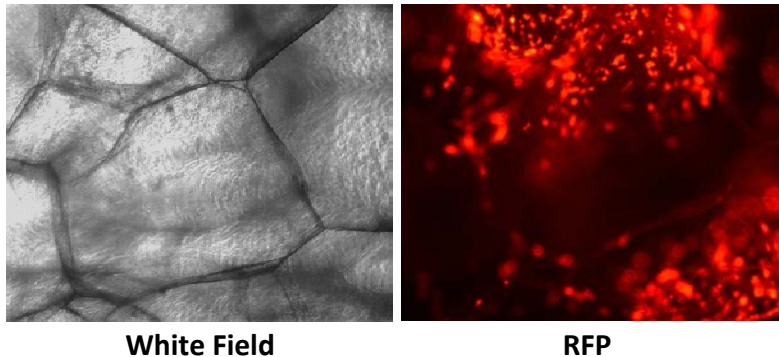
3D-Scaffolds colonization

The third dimension added to cell culture allows higher cell number to be cultured. On a 24 well-plate, up to 200,000 cells can be seeded to colonize a 0.5 x 0.5 x 0.5 cm³ sponge.



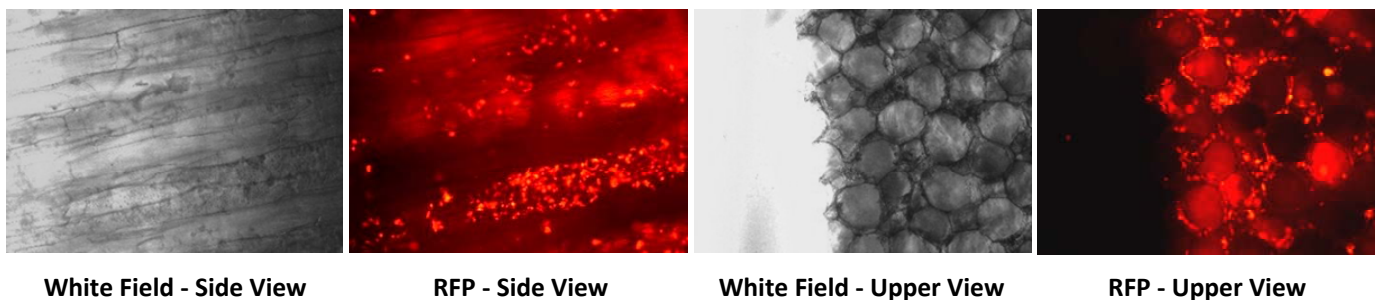
25,000 to 200,000 cells were added on a 0.125 cm³ Collagen-based or Collagen-derived 3D scaffolds. 48H after seeding, total protein quantity was measured using Bradford kit (cat # BA00100). The protein quantity is proportional to the number of colonizing cells on 3D scaffolds. Depending on the scaffold volume, complexity and cell type, numbers of seeded cells may have to be adjusted.

1- Collagen based 3D-scaffolds



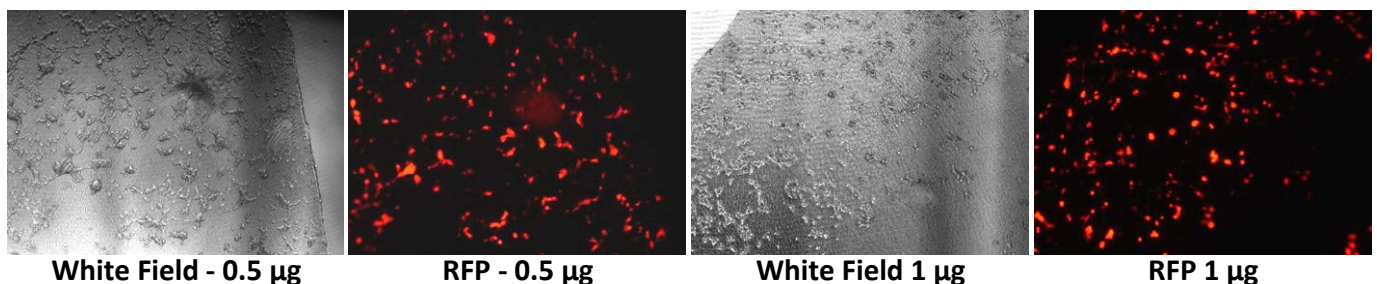
COS-7 cells (0.5×10^5 cells/ well) were transfected in a Collagen-based 3D scaffold preloaded with complexes formed by 3 μg of RFP plasmid DNA and 12 μL of 3D-Fect transfection reagent per well in a 24-well plate. Photos were taken under white field and fluorescence 24h post-transfection.

2- Honeycomb Collagen-derived 3D-scaffolds



HEK-293T cells (0.5×10^5 cells/ well) were transfected in a honeycomb arranged collagen-derived 3D Scaffold preloaded with complexes formed by 3 μg of RFP plasmid DNA and 12 μL of 3D-Fect transfection reagent per well in a 24-well plate. Photos were taken under white field and fluorescence after 24h transfection.

3- Hydrophobic Poly-Tetrafluoroethylene (PTFE) Culture Inserts

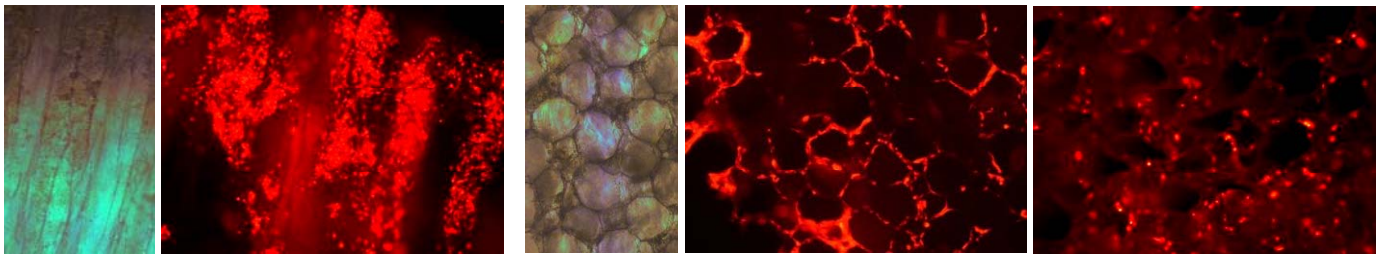


COS-7 cells (0.5×10^5 cells/ well) were transfected on a PTFE Culture Insert preloaded with 3 μg of RFP plasmid DNA and 12 μL of 3D-Fect transfection reagent per well in a 24-well plate. Photos were taken under white field and fluorescence 24h post-transfection.

Table 1: Examples of 3D-Scaffolds successfully tested with **3D-Fect™** Transfection Reagent.

3D Scaffold	
Collagen	Collagen-based Scaffolds
Collagen-derived	Collagen-derived Scaffolds
HA	Hyaluronic Acid
Millicell™ (PTFE)	Cell culture insert (Millipore)
PCL	Polycaprolactone
PEG	Poly(Ethylene Glycol)
PLGA	Poly(lactic-co-glycolic acid)
PS	Poly(Styrene)
PU	Poly(Urethane)

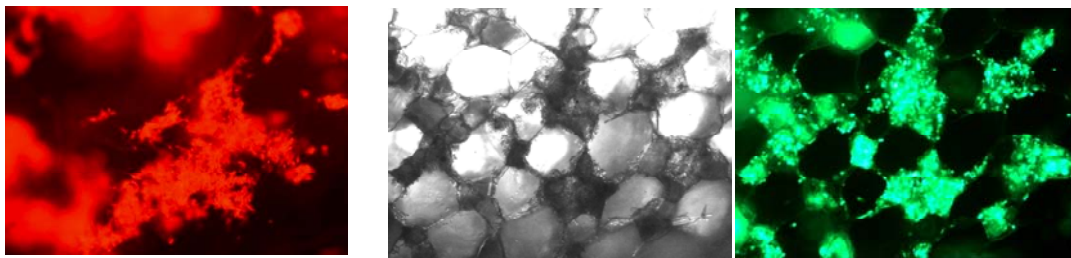
3D-Fect™ transfection efficiency on several cell types



HEK-293T

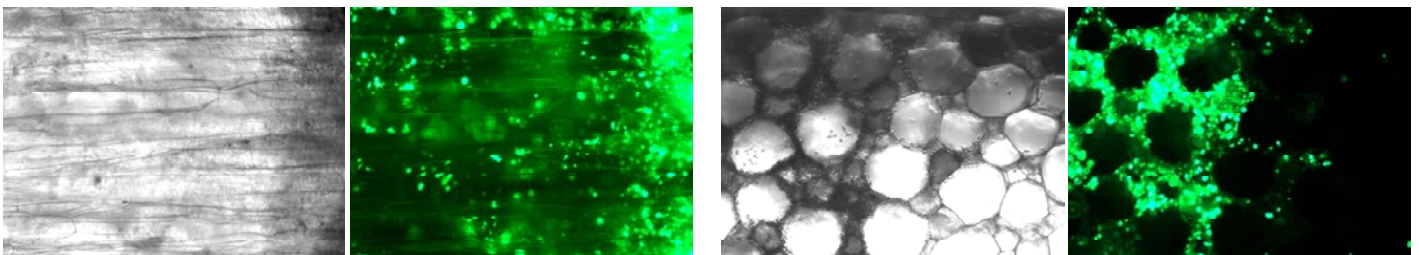
COS-7

NIH-3T3



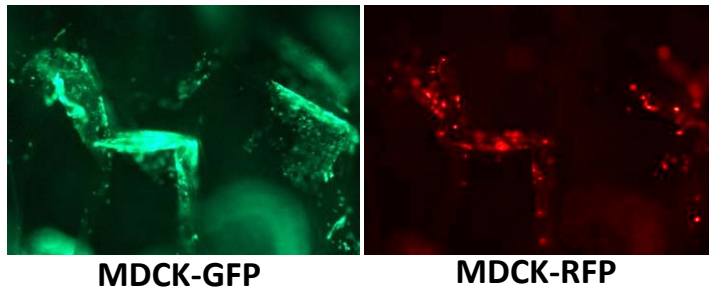
COS-7

COS-7



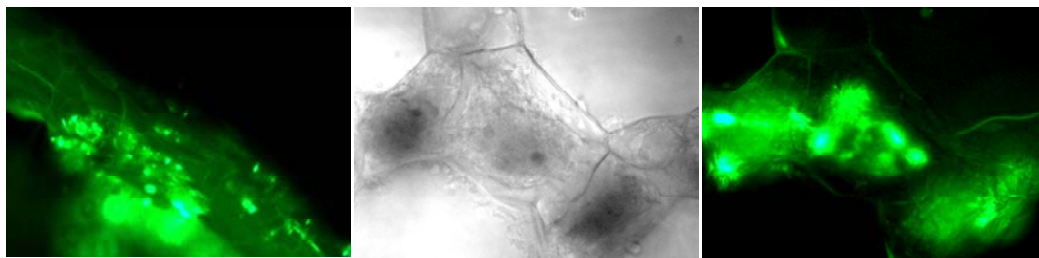
HeLa

HeLa



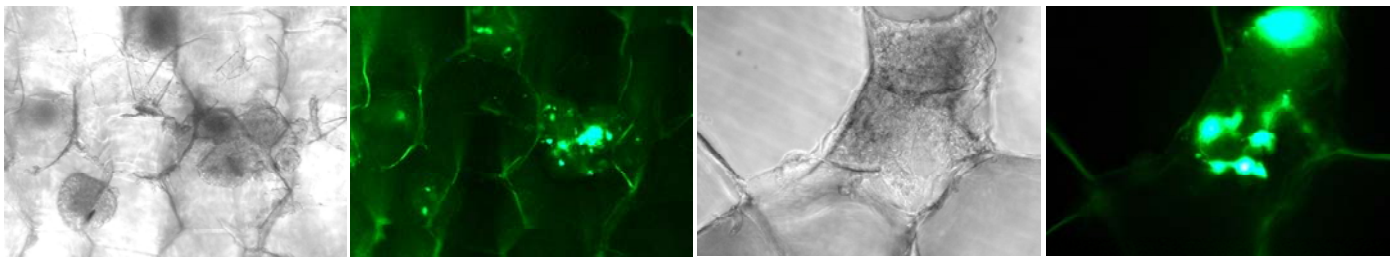
MDCK-GFP

MDCK-RFP



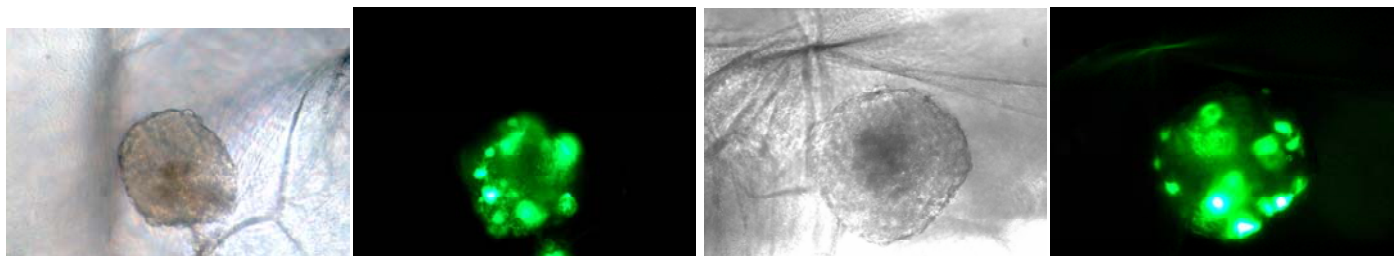
MEF (4X)

Mouse Embryonic Fibroblast - MEF (20X)



Neural Stem Cells - NSC (4X)

Neural Stem Cells - NSC (20X)



NSC (20X)

NSC (20X)

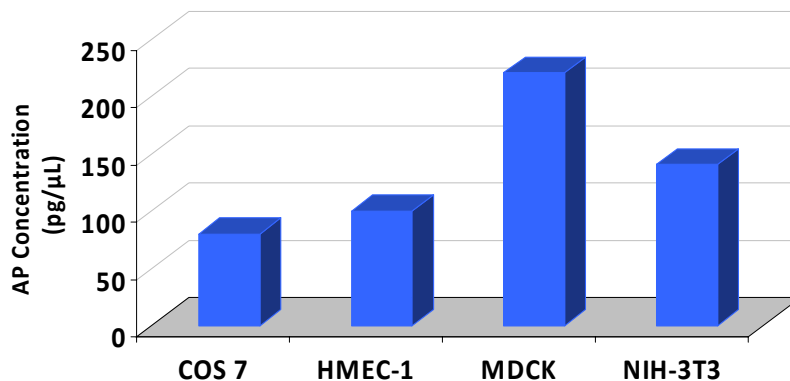
Several cell lines (1×10^5 cells/ well) were transfected in various 3D scaffolds loaded with 3 μg of RFP or GFP plasmid DNA (pVectOZ GFP, # PL00120) complexed to 12 μL of 3D-Fect transfection reagent per well in a 24-well plate. Neural stem cells were transfected with 3 μg DNA and 6 μL of 3D-Fect. Depending on the scaffold composition, the structure and the complexity of the matrix vary; cells will adhere differently from one kind of scaffold to another. Photos were taken under white field and fluorescence 24h post-transfection.

Table 2: Example of cells successfully transfected on 3D Scaffolds with **3D-Fect™** transfection reagent.

Cell Lines	Cell Type	Species	Transgene expression level
293, 293T	Kidney	Human	+++
A549	Non-small cell lung carcinoma	Human	++
BEAS-2B	Bronchial Epithelial	Human	++
CHO, CHO-K1	Ovary (epithelial like)	Chinese Hamster	+++
COS-1 , COS-7	Kidney	Green Monkey	+++
HEK293	Kidney	Human	+++
HeLa, HeLa-S3	Cervix carcinoma	Human	+++
HMEC-1	Microvascular Endothelial	Human	++
MCF-7	Breast adenocarcinoma	Human	++
MDCK	Kidney	Dog	++
MEF	embryonic fibroblasts	Mouse	+++
NSC	Neural stem cells	Mouse	+++
NIH-3T3	Fibroblasts	Mouse	++
RAW	Macrophage	Mouse	+
SH-SY5Y	Neuroblastoma	Human	++
Vero	Kidney	Green Monkey	+++

High secreted protein expression level

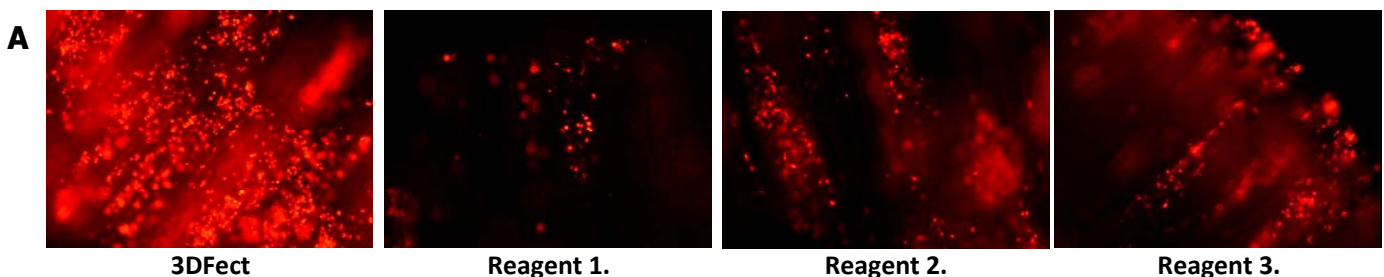
Alkaline Phosphatase (AP) secretion

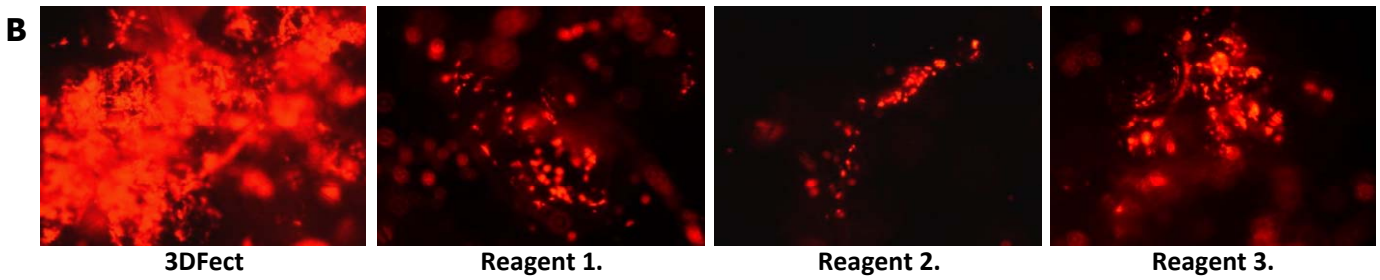


Cells (1×10^5 cells/ well) were transfected in a collagen-based 3D-Scaffold with 3 μ g of SEAP plasmid (pVectOZ SEAP, # PL00150 encoding for secreted human embryonic alkaline phosphatase) and 12 μ L of 3D-Fect per well in a 24-well plate, according to the 3D-Fect protocol. Alkaline phosphatase production was monitored at day 3 post-transfection.

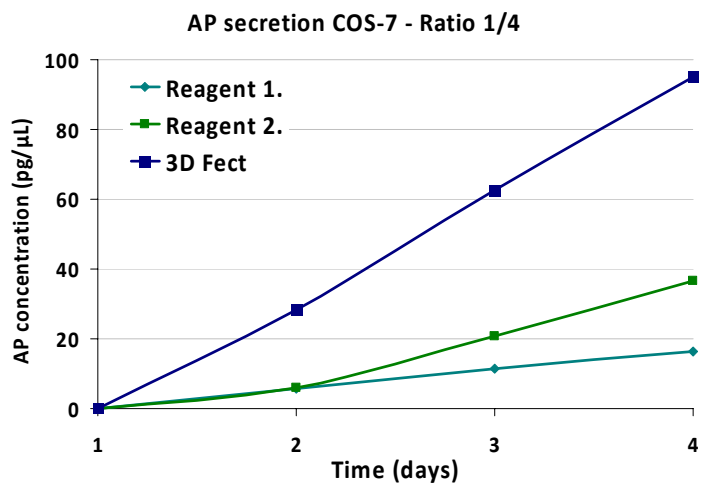
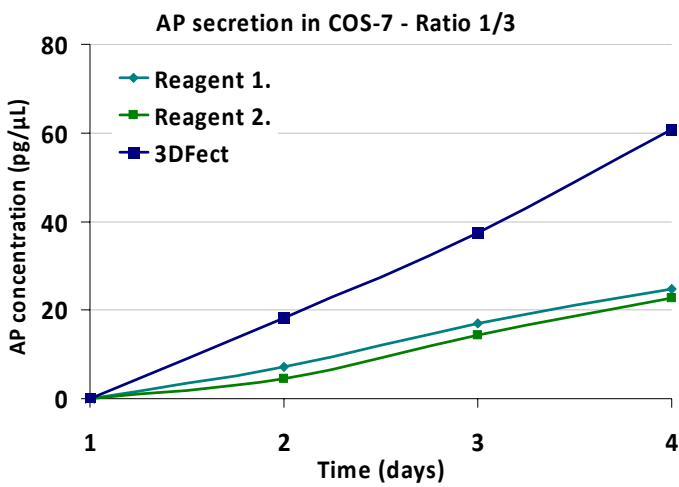
3D-Fect™ outperforms other transfection reagents

3D-Fect™ – comparison with other transfection reagents

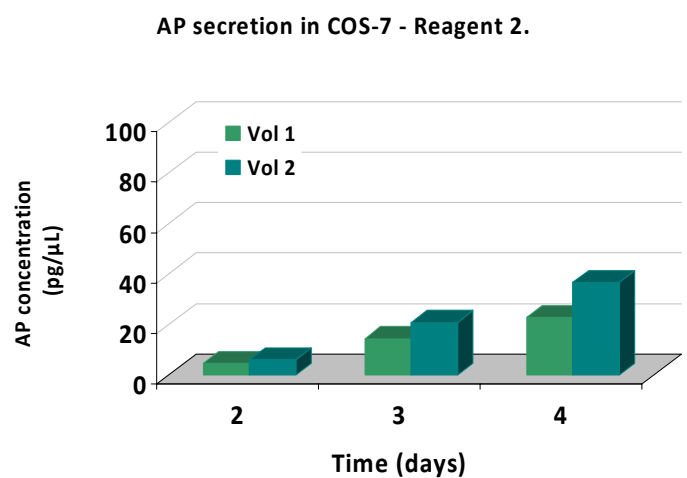
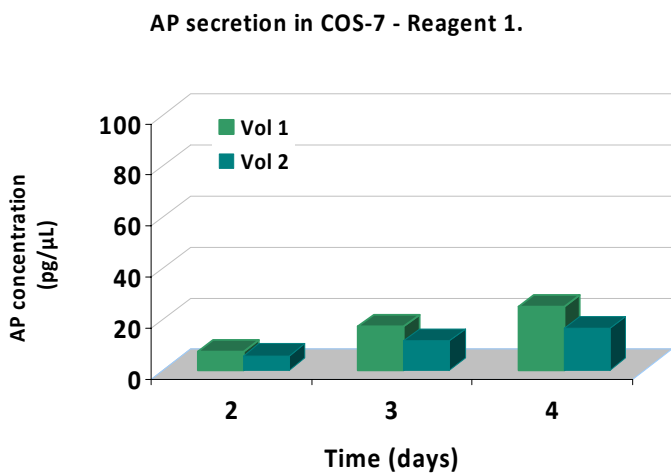




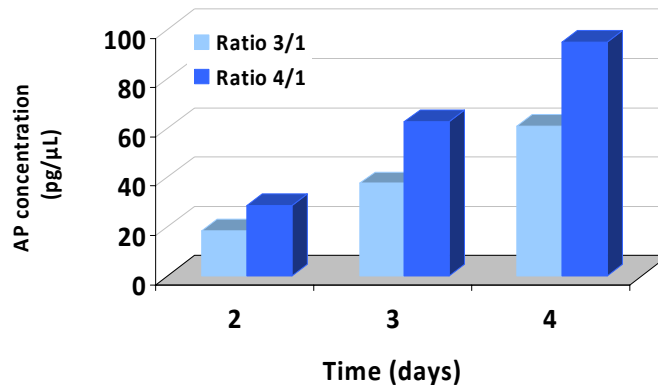
COS-7 and HEK-293T (1×10^5 cells/ well) were transfected in 3D-Scaffolds formed with Collagen (A) or Collagen derivative (B) respectively. Transfections were performed with 3 μg of RFP plasmid and 12 μL of 3D-Fect per well in a 24-well plate as described in the protocol. Other reagents were tested according to the manufacturer's instructions. Fluorescence expression was monitored 48 h post-transfection.



COS-7 (1×10^5 cells/ well) were transfected in a collagen based 3D-Scaffold with 3 μg of SEAP plasmid (pVectOZ SEAP, # PL00150) and 9 or 12 μL of 3D-Fect per well in a 24-well plate (ratio 1/3 and 1/4 respectively) as described in the protocol. The other reagents were tested according to the manufacturer's instructions using two transfection reagent volumes. Alkaline phosphatase production was monitored at day 2, 3 and 4 post-transfection.

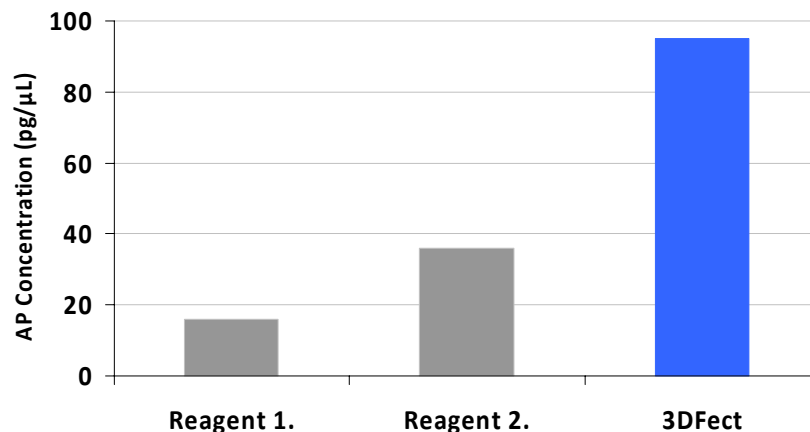


AP secretion in COS-7 - 3DFect.



COS-7 (1×10^5 cells/ well) were transfected in a Collagen based 3D-Scaffold with 3 μ g of SEAP plasmid (pVectOZ SEAP, # PL00150) and 9 or 12 μ L of 3D-Fect per well in a 24-well plate (ratio 1/3 and 1/4 respectively) as described in the protocol. The other reagents were tested according to the manufacturer's instruction using two transfection reagent volumes. Alkaline phosphatase production was monitored at day 2, 3 and 4 after transfection.

Comparison 3DFect Efficiency



COS-7 (1×10^5 cells/ well) were transfected in a collagen based 3D-Scaffold with 3 μ g of SEAP plasmid and 12 μ L of 3DFect per well in a 24-well plate. 3DFect transfections were performed as described in the protocol. The other reagents were tested according to the manufacturer's instruction. Alkaline phosphatase production was monitored at day 4 post-transfection.

Optimization of DNA /3D-Fect™ ratio

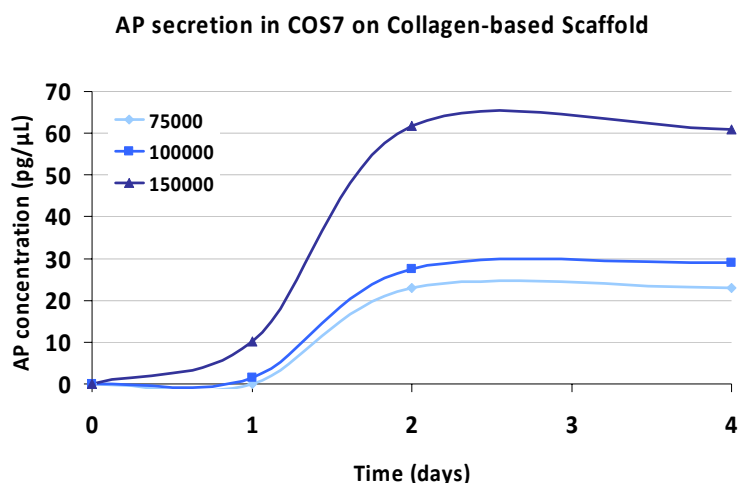
The general protocol is as simple as using **4 μ L of 3D-Fect™ per 1 μ g of DNA**.

However, optimal conditions may vary depending on the nucleic acid, cell type, 3D Scaffold composition and complexity, 3D Scaffold volume and presence or absence of serum. Therefore, the amounts and ratios of the individual components (DNA and 3D-Fect™) may have to be adjusted to achieve best results (see examples of results below). Consequently, we suggest that you optimized these important parameters.

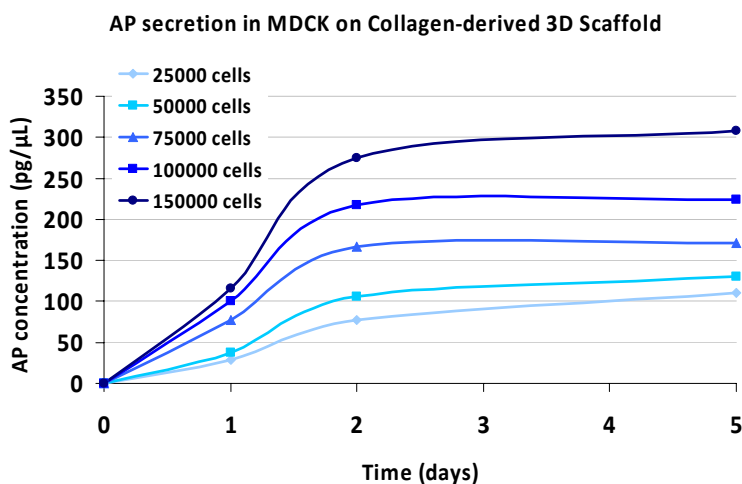
1. The ratio of 3D-Fect™ / DNA
2. The quantity of DNA
3. The cell number
4. The presence or absence of serum
5. The incubation time

Our team has developed many cell type specific protocols with optimized transfection conditions. Please contact our technical support service to obtain these protocols: tech@ozbiosciences.com

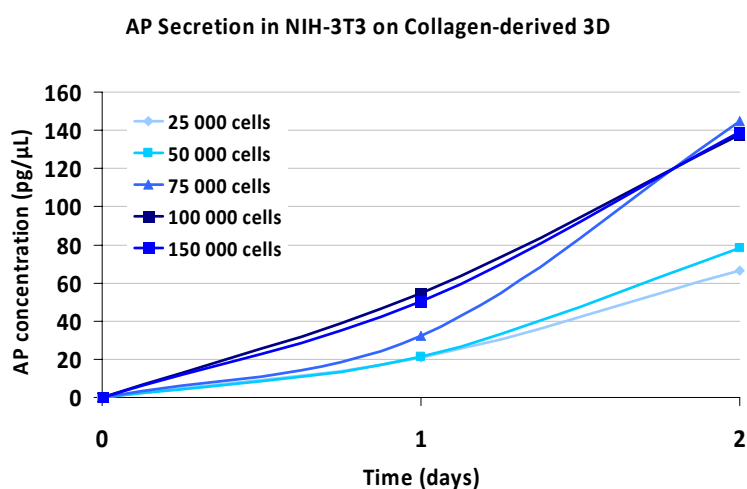
1. Optimization of cell number



Various numbers of COS-7 cells were transfected in Collagen-based 3D-Scaffold with 3 μg of SEAP plasmid and 12 μL of 3D-Fect in a 24-well plate. SEAP secretion was monitored over several days post transfection.



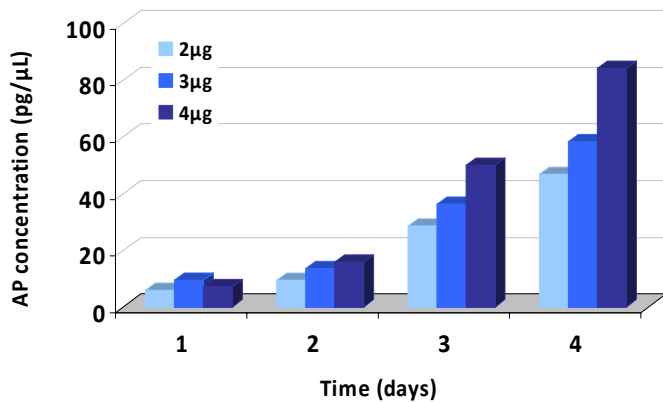
Various numbers of MDCK cells were transfected in Collagen-derived 3D-Scaffold with 3 μg of SEAP plasmid and 12 μL of 3D-Fect in a 24-well plate. SEAP secretion was monitored over several days post transfection.



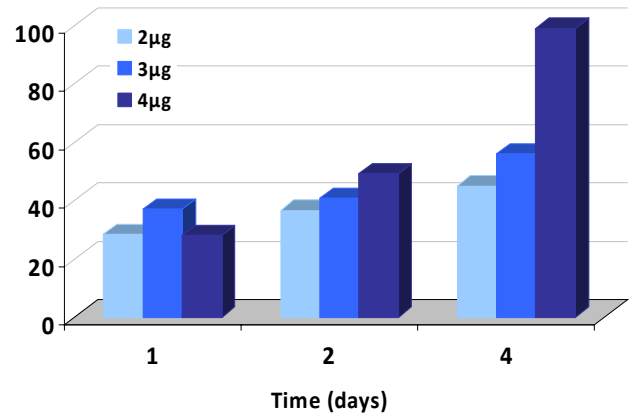
Various numbers of NIH-3T3 cells were transfected in Collagen-derived 3D-Scaffold loaded with 3 μg of SEAP plasmid and 12 μL of 3D-Fect in a 24-well plate. SEAP secretion was monitored 1 and 2 days post transfection.

2. Optimization of DNA amount

AP Secretion in COS 7 on Collagen-derived 3D Scaffold



AP Secretion in HMEC-1 on Collagen-Derived 3D Scaffold



COS-7 and HMEC-1 (1×10^5 cells/ well) were transfected in a Collagen-derived 3D-Scaffold with various amounts of SEAP plasmid and a fixed 3D-Fect ratio per well in a 24-well plate ($4 \mu\text{L}$ per μg DNA). 3D-Fect transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored at day 1, 2, 3 and 4 after transfection.

Bibliographic references

Please consult our list of references available on the website: www.ozbiosciences.com.