3D-FectIN™ transfection reagent - Results

OZ Biosciences is delighted to announce the launching of a new 3D transfection reagent, ***3D-FectIN™***. 3D-FectIN™ is the newest reagent specifically designed and developed for transfection of cells cultured in 3D on hydrogels (collagen, hyaluronic acid, PEG, laminin...). This formulation is based on a novel technology that allows adding a third dimension to cell cultures.

Main ***3D-FectIN™*** features are:

1. Highly efficient
2. Ideal for any gel and hydrogel supporting 3D culture
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. Compatible with multiple applications
9. Long term transgene expression

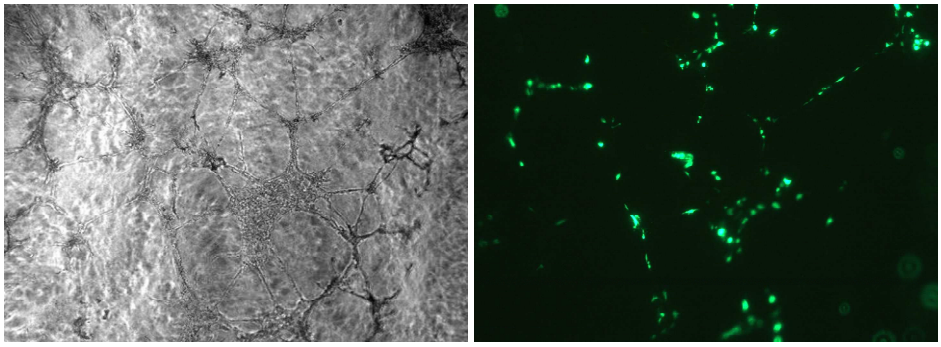
Nucleic acid types

3D-FectIN™ 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 hydrogels

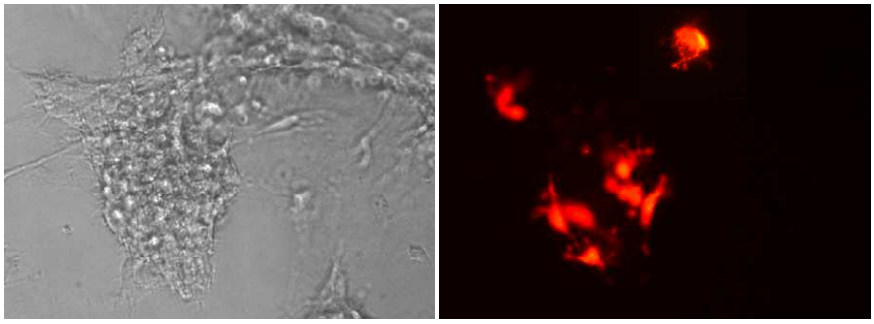
3D-FectIN™ is suitable for numerous cells (see Table 1) and several hydrogel types (see Table 2). If a particular cell type or Hydrogel type is not listed, this does not imply that ***3D-FectIN™*** is not going to work. OZ Biosciences is maintaining an updated list of cells successfully tested available 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-FectIN™ transfection efficiency on several cell types



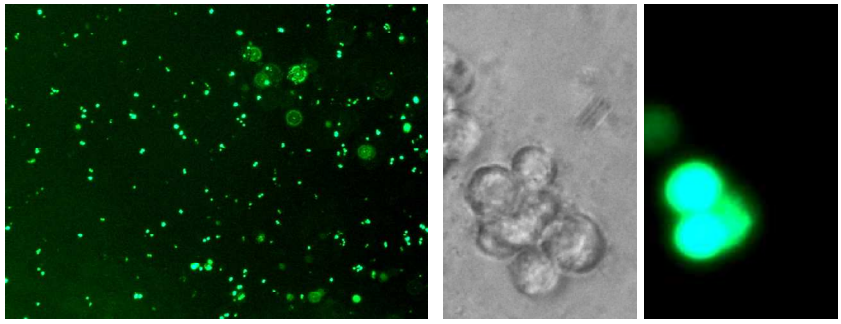
HMEC-1 - White Field **HMEC-1 - GFP**

HMEC-1 (30,000 cells/ well) were transfected in collagen-based hydrogel (Matrigel™*, BD Biosciences) preloaded with complexes formed by 2 µg of GFP plasmid DNA (pVectOZ GFP, # PL00120) and 8 µL of 3D-Fectin transfection reagent per well in a 96-well plate. Photos were taken under white field and fluorescence 48h post-transfection.



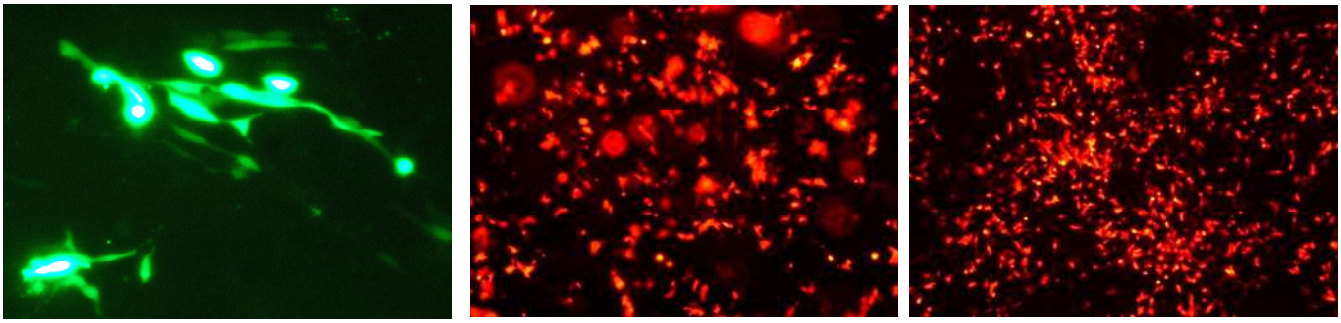
Neural Stem Cells - White Field **Neural Stem Cells - Fluorescence**

Neurospheres composed by Neural Stem Cells (NSC), (20,000 cells/ well) were transfected in collagen-based hydrogel (Matrigel™, BD Biosciences) preloaded with complexes formed by 1 µg of RFP plasmid DNA and 1 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under white field and fluorescence 48h post-transfection.



Raw (4X) **Raw (20X)**

Raw macrophages, (30,000 cells/ well) were transfected in collagen-derived hydrogel preloaded with complexes formed by 2 µg of RFP plasmid DNA and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under white field and fluorescence 48h post-transfection.



NIH-3T3 (20X) **COS-7 (4X)** **HEK-293T (4X)**

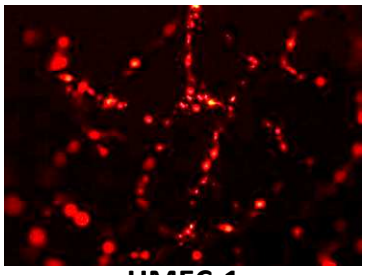
NIH-3T3 Fibroblasts, COS7 and HEK-293T cells (30,000 cells/ well) were transfected in collagen-derived hydrogel preloaded with complexes formed by 2 µg of RFP plasmid DNA (respectively encoding for RFP and GFP protein) and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under fluorescence 48h post-transfection.

Table 1: Example of cells successfully transfected on 3D Scaffolds with ***3D-FectIN™***.

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	++
hASC	Adipocyte Stromal Cells	Human	+
HEK293	Kidney	Human	+++
HeLa, HeLa-S3	Cervix carcinoma	Human	++
HMEC-1	Microvascular Endothelial	Human	++
MCF-7	Breast adenocarcinoma	Human	++
MDCK	Kidney	Dog	++
NIH-3T3	Fibroblasts	Mouse	++
NSC	Neural stem cells	Mouse	+++
RAW	Macrophage	Mouse	+
SH-SY5Y	Neuroblastoma	Human	++
Vero	Kidney	Green Monkey	+++

3D-FectIN™ transfection efficiency in several types of 3D-hydrogels

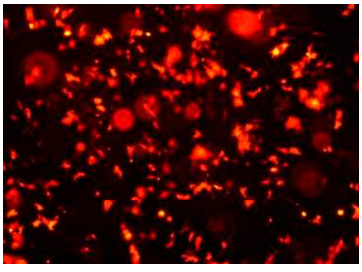
1- Collagen-based hydrogels (Matrigel™, BD Biosciences)



HMEC-1

HMEC-1 (30,000 cells/ well) were transfected in collagen-based hydrogel (Matrigel™, BD Biosciences) preloaded with complexes formed by 2 µg of RFP plasmid DNA and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under fluorescence 48h post-transfection.

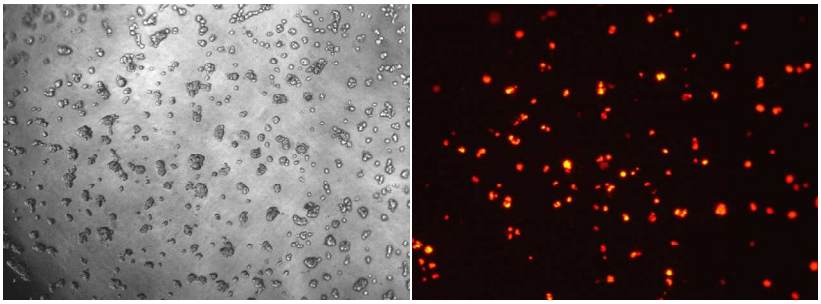
2- Collagen-derived hydrogels



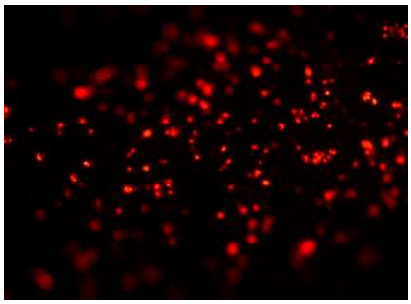
COS 7

COS-7 cells (30,000 cells/ well) were transfected in a collagen-derived hydrogel preloaded with complexes formed by 2 µg of RFP plasmid DNA and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under white field and fluorescence 48h post-transfection.

3- Hyaluronic Acid (HA) -based hydrogels



COS7 - White Field COS7 - Fluorescence



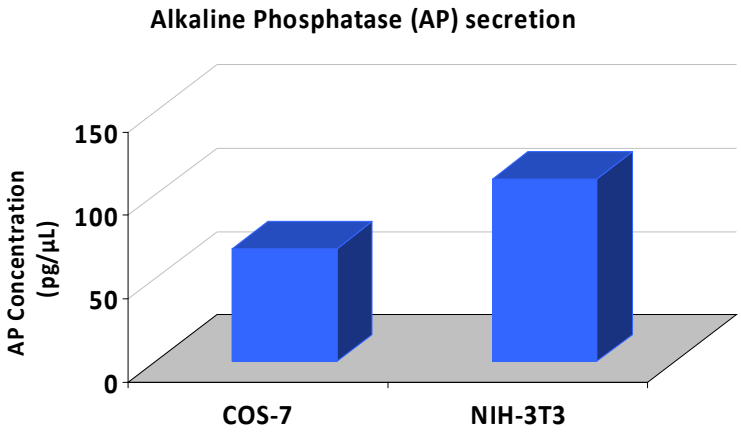
HEK-293 T

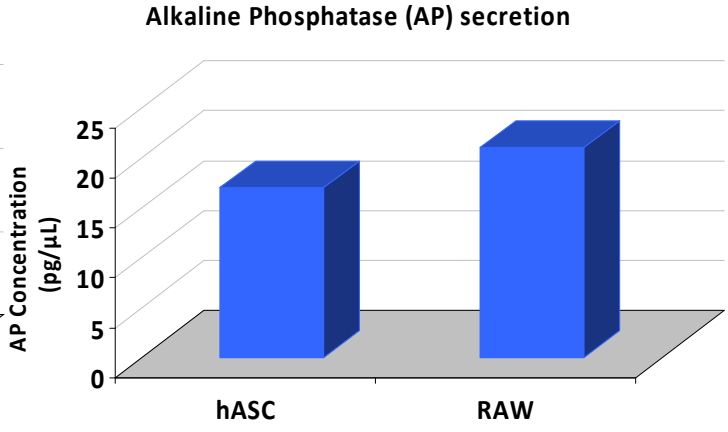
HEK-293T and COS7 cells (10,000 cells/ well) were transfected in hyaluronic acid-based hydrogels preloaded with 3 µg of RFP plasmid DNA and 6 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Photos were taken under white field and fluorescence 48h post-transfection.

Table 2: Examples of hydrogels successfully tested with ***3D-FectIN™*** transfection reagent.

Hydrogels	
Collagen	Collagen-Based Hydrogels
Collagen-Derived	Collagen-Derived Hydrogels
HA	Hyaluronic Acid
Gelatin	Extracellular Matrix (ECM)
Fibrin / Fibronectin	ECM
Fibrinogen	ECM
Laminin	ECM
Matrigel™	BD Bioscience
Poly-(Ethylene Glycol)	PEGylated hydrogels

High secreted protein expression Level

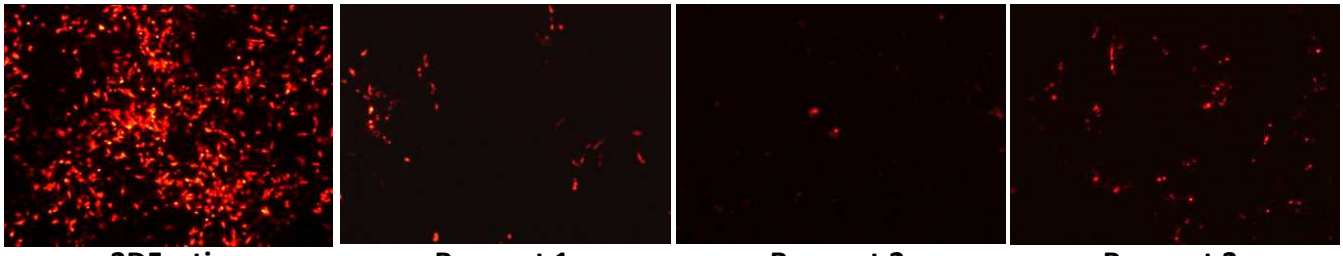




Several cells (30,000 cells/ well) were transfected in a collagen-derived hydrogel preloaded with complexes formed by 2 µg of SEAP plasmid DNA (pVectOZ SEAP, # PL00150 encoding for secreted human embryonic alkaline phosphatase) and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. Alkaline phosphatase secretion in culture medium was monitored at day 2 after transfection.

3D-FectIN™ outperforms other transfection reagents

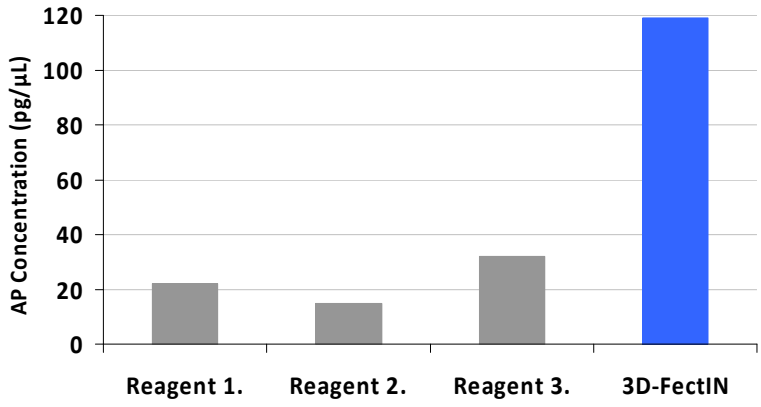
3D-FectIN™ – Comparison with other transfection reagents



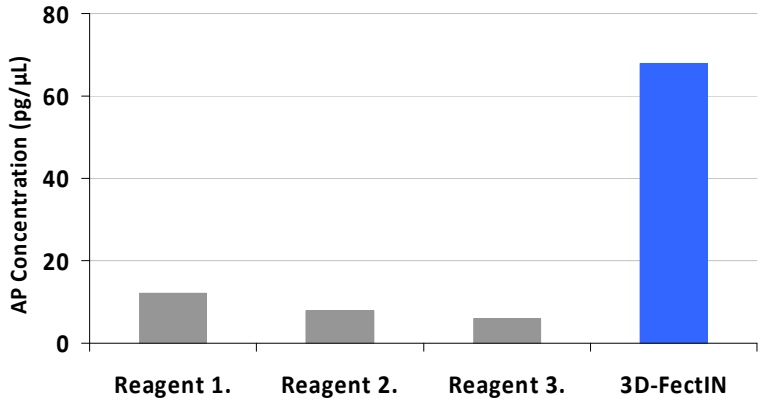
3DFectin **Reagent 1.** **Reagent 2.** **Reagent 3.**

HEK-293T (30,000 cells/ well) were transfected in a collagen-derived hydrogel preloaded with complexes formed by 2 µg of RFP plasmid DNA and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. 3D-FectIN™ transfections were performed as described in the protocol. Other reagents were tested according to the manufacturer's instructions. Fluorescence expression was monitored 48 h post-transfection.

Comparison 3DFectIN Efficiency - NIH-3T3



Comparison 3DFectIN Efficiency - COS-7



NIH-3T3 and COS-7 (30,000 cells/ well) were transfected in a collagen-derived hydrogel preloaded with complexes formed by 2 µg of RFP plasmid DNA and 8 µL of 3D-FectIN™ transfection reagent per well in a 96-well plate. 3D-FectIN™ transfections were performed as described in the protocol. Other reagents were tested according to the manufacturer's instructions. Alkaline phosphatase secretion in culture medium was monitored at day 2 after transfection.

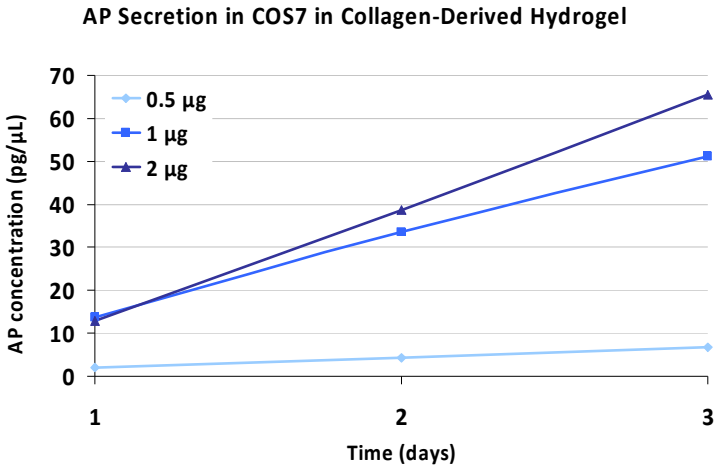
Optimization of DNA /3D-FectIN™ ratio

The general protocol is as simple as follow: **use 4 µL of 3D-FectIN™ per 1 µg of DNA.**
However, optimal conditions may vary depending on the nucleic acid, cell type, hydrogel composition and complexity, hydrogel volume and presence or absence of serum. Therefore, the amounts and ratios of the individual components (DNA and 3D-FectIN™) may have to be adjusted to achieve best results (see examples of results below). Consequently, we suggest that you optimized these important parameters.

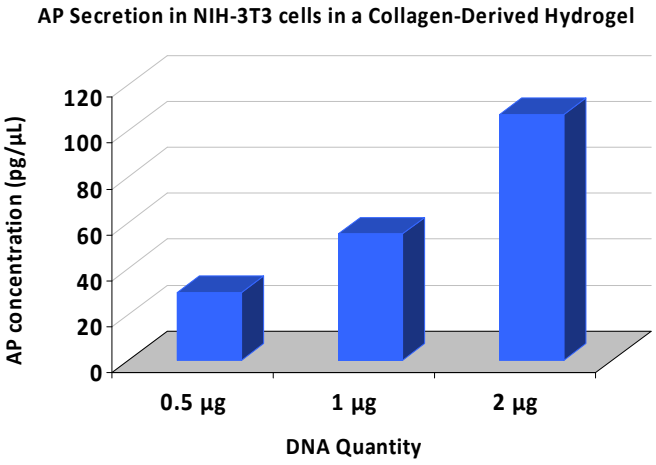
1. The ratio of 3D-Fectin / DNA
2. The quantity of DNA
3. The cell number
4. The presence or absence of serum
5. The incubation time

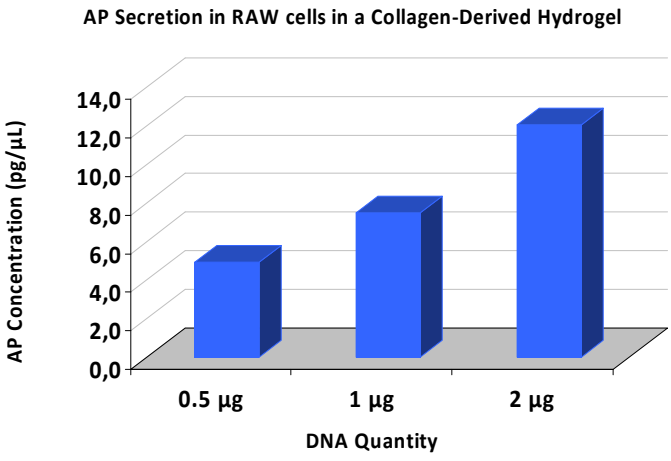
Our team has developed many cell type specific protocols with optimized transfection conditions. Please contact our technical support service to obtain these protocols: tech@ozbiosciences.com

1. Optimization of DNA amount



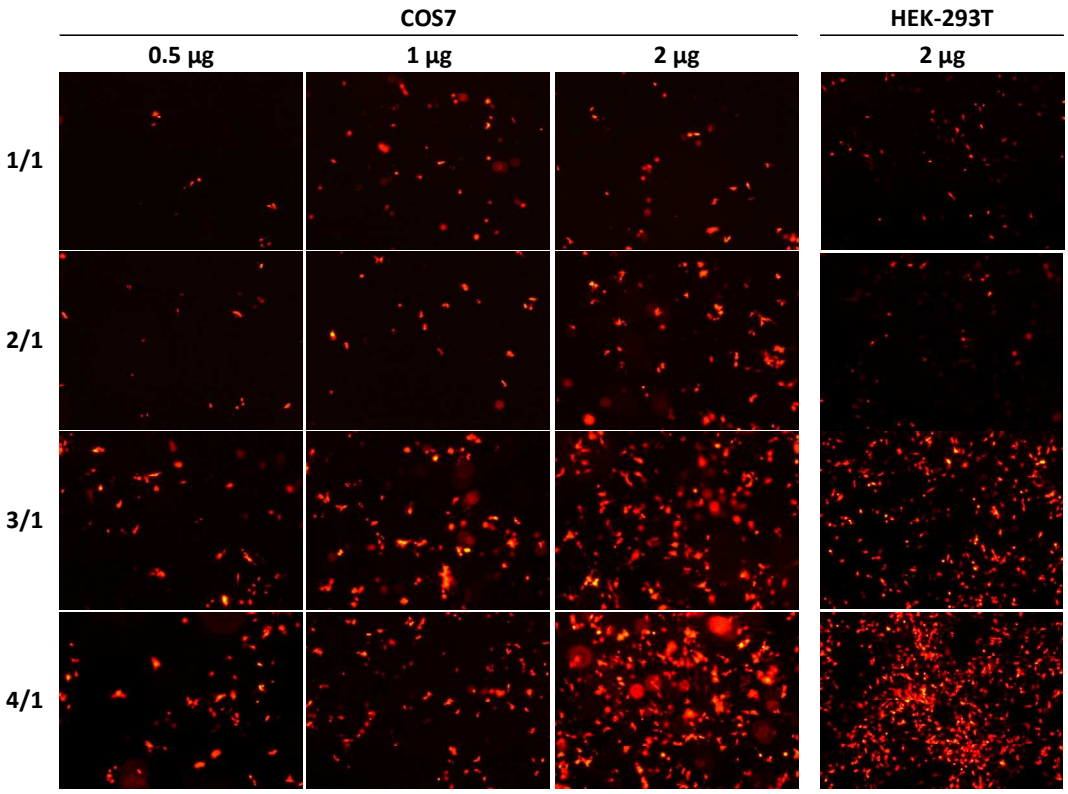
COS-7 cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogel with various amounts of SEAP plasmid and a fixed 3D-FectIN™/DNA ratio per well in a 96-well plate (4µL per µg DNA). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored at day 1, 2 and 3 after transfection.



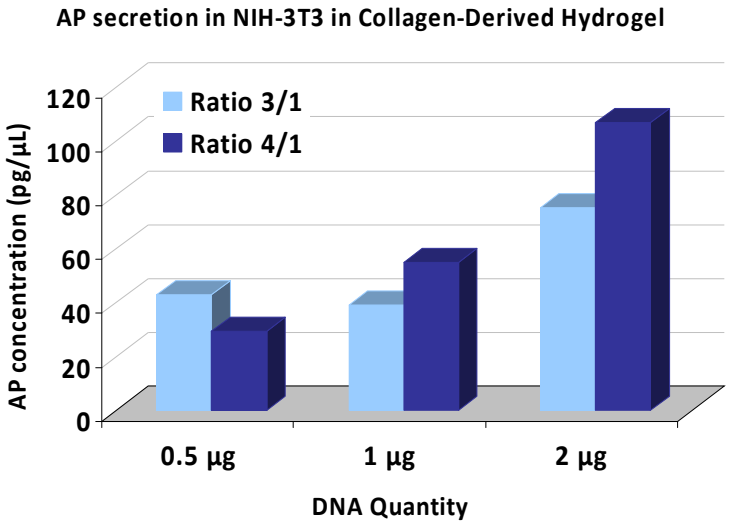


NIH-3T3 and Raw cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogel with various amounts of SEAP plasmid and a fixed 3D-FectIN™/DNA ratio per well in a 96-well plate (4µL per µg DNA). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored 1 day post-transfection.

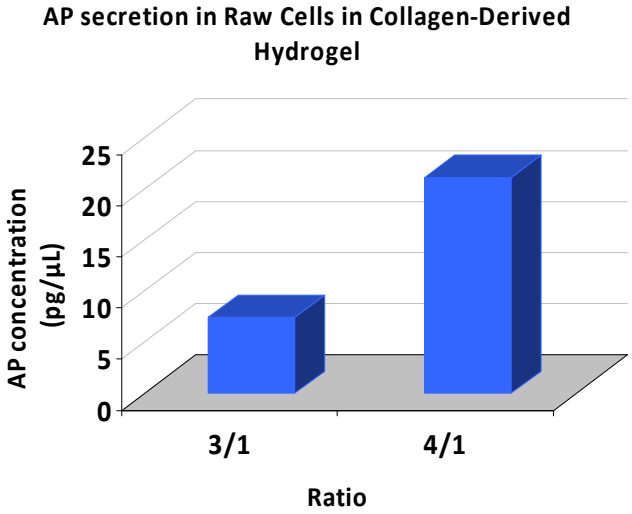
2. Optimization of 3D-FectIN™ ratio



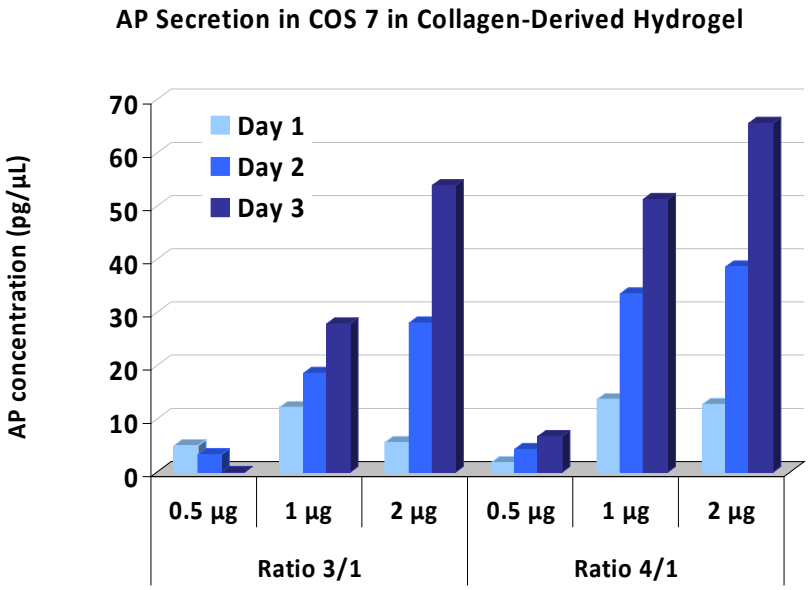
COS7 and HEK-293T cells (30,000 cells/ well) were transfected in a collagen-derived hydrogel preloaded with complexes formed with several volumes of 3D-FectIN™ reagent per well in a 96-well plate (ratio 1/1 to 4/1) and with 0.5 to 2 µg of RFP plasmid DNA. 3D-FectIN™ transfections were performed as described in the protocol. Fluorescence expression was monitored 48 h post-transfection.



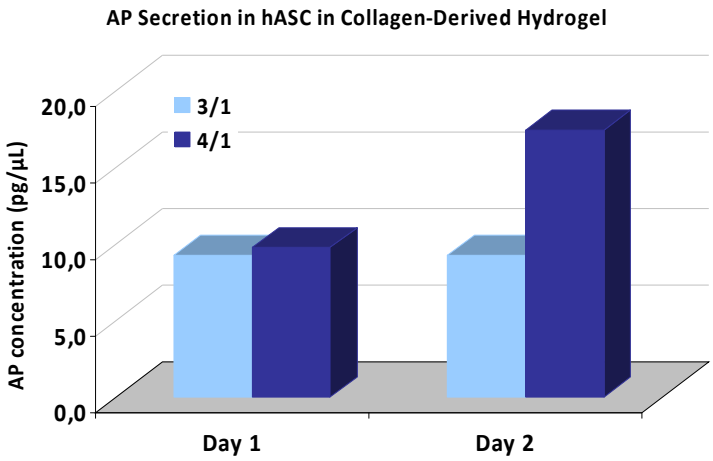
NIH-3T3 cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogel with various amounts of SEAP plasmid and various 3D-FectIN™ /DNA ratio per well in a 96-well plate (3 and 4 µL per µg DNA respectively). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored 2 days post-transfection.



RAW cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogelwith 2 µg of SEAP plasmid and various 3D-FectIN™ /DNA ratio per well in a 96-well plate (3 and 4 µL per µg DNA respectively). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored 1 day after transfection.



COS-7 cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogel with various amount of SEAP plasmid and various 3D-FectIN™ / DNA ratio per well in a 96-well plate (3 and 4 µL per µg DNA respectively). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored at various days after transfection.



hASC (Human Adipocyte Stromal) cells (30,000 cells/ well) were transfected in a collagen-derived 3D-hydrogel with 2 µg of SEAP plasmid and various 3D-FectIN™ /DNA ratio per well in a 96-well plate (3 and 4 µL per µg DNA respectively). 3D-FectIN™ transfections were performed as described in the protocol. Alkaline phosphatase secretion was monitored at day 1 and 2 after transfection.

Bibliographic references

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

* Matrigel™ is a trademark own by BD Biosciences.