

# Transfection

## ExGen 500 Universal Transfection Reagent

22kDa linear PEI for *in vitro* / *in vivo* transfection



**Fermentas**  
LIFE SCIENCES

# Ready-to-use, one-step transfection

**ExGen 500, a 22kDa linear polyethylenimine, belongs to a new class of exceptionally efficient, non-viral, non-liposomal, gene-delivery reagents.**

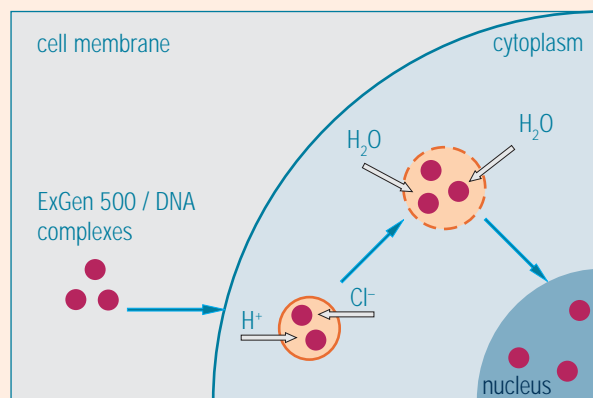
## Universal transfection reagent

- High transfection efficiency of a wide variety of cell types.
- Suitable for:
  - oligonucleotide and plasmid DNA transfections,
  - co-transfections,
  - primary and secondary cell lines,
  - adherent and suspension cells,
  - differentiated and undifferentiated cells,
  - stem cells,
  - stable and transient transfections.
- Excellent transfection efficiency in the presence or absence of serum.
- Minimal cytotoxicity.
- High reproducibility.

## User-friendly transfection reagent

- Ready-to-use (no need to reconstitute, dilute or manipulate) reagent:
  - add DNA to 150mM NaCl,
  - add ExGen 500,
  - incubate 10 minutes at room temperature,
  - add to cells.
- Fast, one step transfection with no need to change media in most cases.
- Economical.

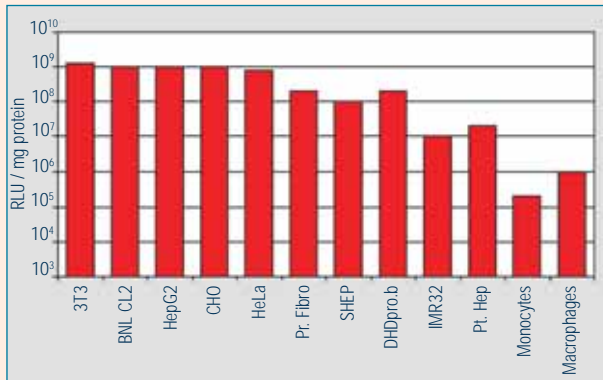
## Mechanism of action



1. ExGen 500 interacts with DNA to form small, stable, highly diffusible complexes which are readily endocytosed.
2. "Proton-sponge" effect of ExGen 500 buffers endosomal pH by provoking massive proton accumulation and passive chloride influx.
3. Rapid osmotic swelling causes endosomal rupture, allowing translocation of DNA to the nucleus without DNA degradation.

# An exceptionally efficient transfection reagent

## ExGen 500 transfects a wide variety of cell types

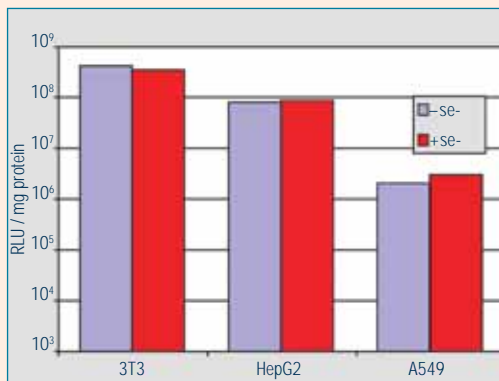


### Transfection of different cell lines with pCMV-Luc complexed with ExGen 500.

Expression of pCMV-Luc was measured in relative light units (RLU)/mg protein.

**Ref.** Boussif, O., Thèse de doctorat, ULP, Strasbourg, 1996.

## ExGen 500 high transfection efficiency is not affected by serum



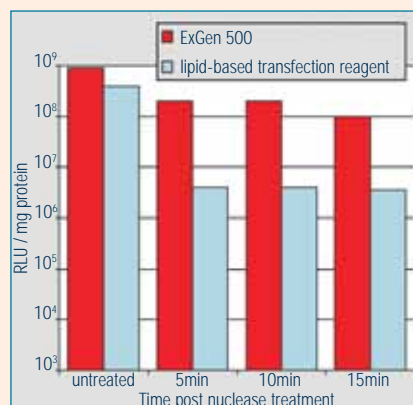
### Transfection of 3T3, HepG2 or A549 cells was performed in the presence or absence of serum.

Expression of pCLuc was measured in relative light units (RLU)/mg protein. The presence of serum did not affect the high transfection efficiency of ExGen 500.

## ExGen 500 protects DNA from nuclease degradation

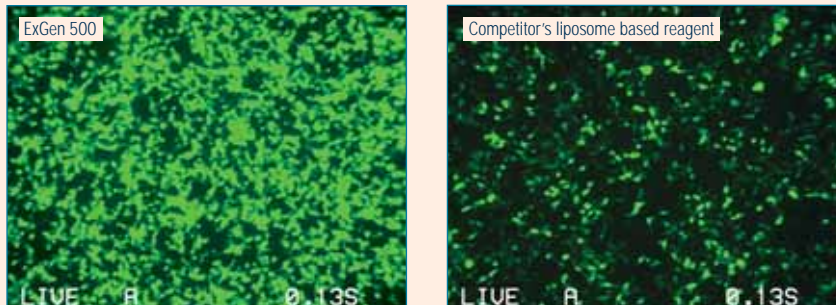
pCMV-Luc was complexed with ExGen 500 or lipid-based transfection reagent. The complexes were treated with DNase I for the time specified. Transfection was carried out on 3T3 cells in triplicate. Expression of pCLuc was measured in relative light units (RLU)/mg protein. ExGen 500 showed greater protection of DNA from nuclease degradation.

**Ref.** Ferrari, S., et al, Biochim Biophys Acta, 1447(2-3): 219-25, 1999.



## High reproducibility – even in co-transfection

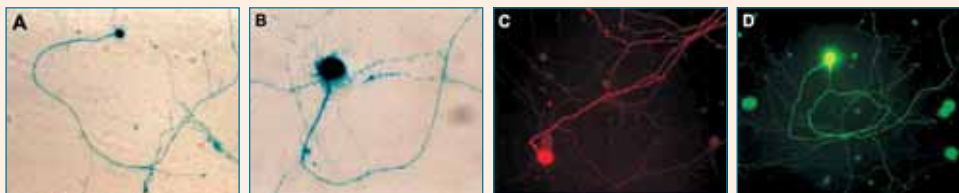
### ExGen 500 performs when other transfection reagents fail



#### Expression of Green Fluorescent Protein (GFP) in 293 cells.

Cells were transfected with a vector containing the GFP coding sequence using ExGen 500 and competitor's liposome based reagent.

### ExGen 500 is effective even in hard-to-transfect cell lines



#### ExGen 500 used to express $\beta$ -galactosidase (A, B, C) and eGFP (D) in postmitotic rat sympathetic neurons.

A – low magnification, stained with X-Gal,  
B – high magnification, stained with X-Gal,

C – immunostained with antibody to  $\beta$ -gal,  
D – eGFP expression.

Note the extensive expression throughout neurons, including synaptic varicosities and grown cells.

$\beta$ -gal expression was detected at 6h post-transfection, with maximum expression (~9% neurons) at 3 days. 95 of 100 cells expressing eGFP were neuronal cells as defined by immunostaining with the neuron-specific marker MAP2.

### Reliable co-transfection



#### Co-transfection of perinatal rat SCG neurons with plasmids expressing $\beta$ -galactosidase and eGFP using Exgen 500.

A – phase contrast image, C – eGFP expression.  
B –  $\beta$ -galactosidase expression,

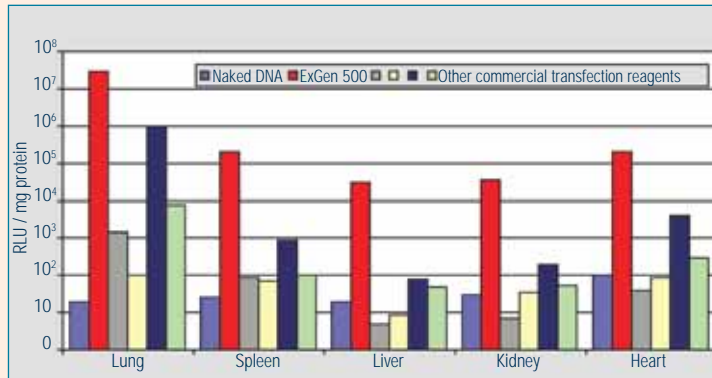
Plasmids were co-transfected in a 1:1 ratio.

23 of 24 transfected neurons examined clearly demonstrated expression of both plasmids.

**Ref.** Horbinski, C., et al, BMC Neuroscience, 2:2, 2001. <http://www.biomedcentral.com/1471-2202/2/2>

# Superior *in vivo* transfection

## ExGen 500 is a superior *in vivo* transfection reagent in all organs tested



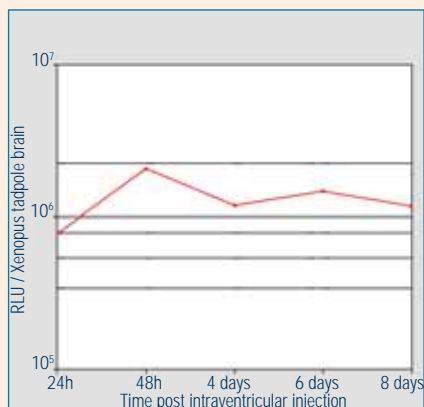
### Intravenous Luciferase gene transfer efficiencies using ExGen 500 and other commercial transfection reagents.

pCLuc was complexed with ExGen 500 or other commercial gene-delivery reagents using the optimal conditions suggested by the manufacturers' protocols. Mice were sacrificed 24h post-injection and luciferase gene expression in various organs was measured in relative light units (RLU). ExGen 500 showed the highest efficiency in all organs.

**Ref.** Bragonzi, A., et al, Gene Ther., Dec;6(12), 1995-2004, 1999.

## ExGen 500 delivers DNA via intravenous, intraventricular (brain), subcutaneous tracheal and intraperitoneal injection

### ExGen 500 is gentle on cells and animals



### Intraventricular delivery of pCMV Luciferase gene in Xenopus tadpole using ExGen 500.

pCMVLuc (1µg) was complexed with ExGen 500 in 5% glucose solution and injected into the third ventricle of Xenopus tadpole brain. Animals were sacrificed at the time indicated. ExGen 500 showed exceptional gene delivery to the central nervous system over 8 days, while at the same time allowing normal tadpole development to occur.

**Ref.** Ouatas, T., et al, Int. J. Dev. Biol., Nov;42(8), 1159-64, 1998.

# The only transfection reagent you need

## Among others, cells successfully transfected with ExGen 500 include:

### Permanently growing cell lines

<b>56FHT80</b>	Human	Fetal tracheal epithelium
<b>6CFSMEo<sup>-</sup></b>	Human	Submucosal gland epithelium cells
<b>9HTEo<sup>-</sup></b>	Human	Normal adult trachea epithelium cells
<b>A549</b>	Human	Type II pneumocytes
<b>B16F10</b>	Mouse	Melanoma cells
<b>BNL CL.2</b>	Mouse	Hepatocytes
<b>C-26</b>	Mouse	Colon carcinoma cells
<b>C2C12</b>	Mouse	Myoblasts
<b>Caco-2</b>	Human	Colorectal adenocarcinoma cells
<b>CFNPE9o<sup>-</sup></b>	Human	Nasal epithelium cells
<b>CFPEo<sup>-</sup></b>	Human	Trachea epithelium cells
<b>Cos-7</b>	Monkey	African green monkey kidney cells
<b>CT26</b>	Mouse	Colon carcinoma cells
<b>HCS-2/8</b>	Human	Chondrocyte-like cells
<b>HeLa</b>	Human	Cervix epitheloid caecinoma cells
<b>Hep 2C</b>	Human	Epidermal carcinoma cells
<b>HepG2</b>	Human	Hepatoma cells
<b>HL-60</b>	Human	Myelomonocytic cell line
<b>J-774</b>	Mouse	Myelomonocytic cell line
<b>Jurkat</b>	Human	T cell leukemia
<b>KB</b>	Human	Epithelial cells
<b>KBv</b>	Human	Drug-resistant derivative of KB
<b>L929</b>	Mouse	Subcutaneous connective tissue fibroblasts
<b>LLC-MDR1</b>	Porcine	Drug-resistant derivative of LLC-PK1
<b>LLC-PK1</b>	Porcine	Kidney epithelial cells
<b>MCA-38</b>	Mouse	Colon carcinoma cells
<b>MCF7</b>	Human	Breast adenocarcinoma cells
<b>MCF7 ADR</b>	Human	Drug-resistant derivative of MCF7
<b>Neuro2a</b>	Mouse	Neuroblastoma cells
<b>NIH 3T3</b>	Mouse	Embryonic fibroblasts
<b>THP-1</b>	Human	Myelomonocytic cell line
<b>U-937</b>	Human	Myelomonocytic cell line

### Primary cell cultures of:

<i>Tupaia belangeri</i>	Hepatocytes
Pekin Duck	
Human, Rat	Postmitotic neurons
Human	Monocytes / macrophages
Newborn Rats	Dorsal root ganglia neurons

ExGen 500 is supplied as a sterile solution of linear 22kDa polyethylenimine (PEI) in water.

The solution is acidic and stable at 4°C.

#### Ordering Information:

ExGen 500 *in vitro* Transfection Reagent  
#R0511 1.0ml (delivers approx. 300µg DNA at N/P=6)

ExGen 500 *in vivo* Transfection Reagent  
#R0521 0.1ml (delivers approx. 600µg DNA at N/P=6)