

SAINT-MIX DNA - Delivery System

- Cat. No: SM-1001-01: 1mL package includes:** 1 vial with 1mL SAINT-MIX and 1 vial with 12mL Hepes Buffered Salt pH 7.4
- Cat. No: SM-1001-02: 2mL package includes:** 1 vial with 2mL SAINT-MIX and 2 vials with 12mL Hepes Buffered Salt pH 7.4
- Cat. No: SM-1001-04: 4mL package includes:** 2 vials with 2mL SAINT-MIX each and 3 vials with 15mL Hepes Buffered Salt pH 7.4

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1. Introduction

The transfection of DNA has become a tool of major importance in studying the function and regulation of genes *in vitro* as well as *in vivo*. Besides the *in vitro* research approach, more and more interest arises for eventual *in vivo* application of genes, RNA, antisense oligonucleotides and proteins. Electroporation, calcium phosphate co precipitation, and use of viral vectors are the more common transfection techniques. These methods have shown variable success rates when attempting to transfect several varieties of cells. Due to immunogenicity and/or non-compatibility with serum however, the clinical use of these techniques is rather limited or non-existent.

An important step forward is the development of amphiphiles as gene/protein delivery systems.

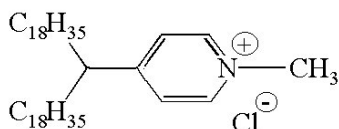
SAINT, is a patented synthetic amphiphilic (non-liposomal) delivery system.

This pyridinium surfactant enables non-toxic delivery of up to 111kb DNA (linear or plasmid), RNA, antisense oligonucleotides and proteins. SAINT/ DNA complexes have been successfully used in several animal models. Transfection can easily be achieved in the presence or absence of serum (2).

2. Product Description/Stability

Formulation: The SAINT-MIX delivery system is based on a cationic pyridinium head group, showing excellent bio-compatibility.

Molecular structure of SAINT-18 (1-methyl-4-(cis-9-dioleyl)methyl-pyridinium-chloride) :



SAINT-MIX consists of SAINT-18 and DOPE (Dioleoyl-phosphatidylethanolamine) dissolved in H₂O. The SAINT-18: DOPE ratio is 1:1.

Stability: SAINT-MIX is supplied in H₂O, 'ready to use', and recommendedly stored at 4° – 7° C. SAINT-MIX does not need to be cooled during experiments, in fact, prolonged exposure (6 months) to ambient temperature did not effect transfection efficacies. Stability experiments have shown that SAINT-MIX stored at the recommended temperature is stable for at least one year after opening.

No loss of function appeared after repeatedly opening of the vials as long as the vials are recapped tightly after use. The antibiotic properties of SAINT-MIX ensure sterility for prolonged periods.

3. Quality Control

Functionality: The functionality assay of SAINT-MIX is performed on HeLa cells. Therefore, SAINT-MIX is tested with a GFP reporter plasmid.

Cytotoxicity: SAINT-MIX has no visible bacterial and fungal contaminations in DMEM cell culture media supplemented with 10% FBS.

4. Applications

A wide variety of mammalian cells can be transfected with SAINT-MIX, including poor transfectable cell-lines such as primary endothelial cells (HUVEC, HUAEC, H5V).

Efficiencies of 15% up to 50% have been achieved with minimal cellular damage. SAINT-MIX has been used to transfect several animal and human cell-types/ lines, some with an **efficiency up to 100%**.

Please consult our website for the latest stability and transfection results: www.synvolux.com

5. Instructions

5.1. General considerations

Transfection-efficiency depends upon the following basic parameters:

- Optimized working concentration of the SAINT-MIX/ DNA complex
- Purity and concentration of nucleic acids
- Cell culture (cell type , cell cycle, etc.)
- Verification of vector construct (promoter, poly(A)-tail, codon usage, etc.)
- Protein expression (time dependent expression levels, toxicity of expressed protein, etc.)

5.2. Recommended concentrations

In general, starting with a concentration of 15nmol SAINT-MIX complexed with 1µg plasmid DNA gives a good transfection result.

A standard pipetting scheme depending on the used culture dishes is depicted underneath. The choice of the well size is dependent on cell type and the preferred method of analysis after transfection.

SAINT-MIX transfection pipetting scheme for several culture dishes

	Petridish 100mm	6 well	12 well	24 well	48 well	96 well
SAINT-MIX (µL)	80	20	10	5	2.5	1.25
HBS (µL)	320	80	40	20	10	5
DNA * (µg)	4	1	0.5	0.25	0.125	0.063
VOLUME (µL)	400	100	50	25	12.5	6.25
SAINT-MIX (nmol)	60	15	7.5	3.75	1.875	0.937

* DNA is diluted at 1µg per 100µL HBS

The DNA / SAINT-MIX ratio could be optimized. Depending on the DNA purity, plasmid length or GC content, a slightly different ratio might improve the transfection efficiency. An example for an optimization scheme in 6-well plates/ 35mm dishes is shown beneath. Other optimization schemes are present at www.synvolux.com

Example for an optimization in 6-well plates:

DNA * (µg)	VOLUME (µL)	10µL	20µL	30µL	40µL
		SAINT-MIX 90µL HBS	SAINT-MIX 80µL HBS	SAINT-MIX 70µL HBS	SAINT-MIX 60µL HBS
0.5	50	A	D	G	J
1	100	B	E	H	K
2	200	C	F	I	L
SAINT-MIX (nmol)		7.5	15	22.5	30

* DNA is diluted at 1µg per 100µL HBS

Example E:

20µL SAINT-MIX (filled up with 80µL HBS) complexed with **1µg** of DNA in 100µL HBS.

The endvolume of SAINT-MIX/ DNA complex is 200µL.

Fill this complex up to 1mL with medium of your choice and add it to the cells.

5.3. Handling instructions

Generally an amount of 1µg DNA complexed with 20µl SAINT-MIX gives a good transfection result in a 35mm dish/ 6-well.

5.3.1. Materials

Before use, SAINT-MIX should be vortexed thoroughly to minimize micelle-size, this will increase complexing efficacy.

HBS is 20mM Hepes and 150mM NaCl, pH 7.4, (included in package). Other buffers that can be used are HBSS (Hanks Buffered Salt Solution) or OptiMEM.

5.3.2. Preparation of SAINT-MIX/ DNA complex for 6-well:

- Apply desired quantity of SAINT-MIX and fill up to 100µL with HBS (included in package), HBSS or OptiMEM.
- Add 0.5 - 2µg plasmid DNA to separate tube and fill up to 100µL with HBS (included in package), HBSS or OptiMEM. Leave to incubate during 5 minutes.
- Pipette the DNA/ HBS solution into the SAINT-MIX/ HBS solution and add this SAINT-MIX/ DNA complex preferably directly or at least within 15 minutes to the cells (Newsletter vol.2 no.2 April 2003). Do not vortex the complex!
- Fill up to 1mL with medium of your choice.

5.3.3. Transfection Protocol:

One day before transfection: Seed a **6-well** plate or **35mm** dish with 1-3.10⁵ cells/ well. Transfection should be performed when cells are 50- 80% confluent.

- Cells are to be washed twice with HBS, HBSS or OptiMEM Some cell-lines showed better transfection results without washing
- Add the prepared SAINT-MIX/ DNA complex to each well and incubate 3 to 4 hours at 37°C
- Add 2mL medium, supplemented with serum, after 3 to 4 hours (leave SAINT-MIX/ DNA complex on the cells).
- After 24-48 hours either refresh your medium, and/ or perform your assay.

Notes:

- Before each transfection new complex should be prepared and used immediately.
- The efficiency of SAINT-MIX for transfection is not influenced by serum (2).

6 Biosafety

It is shown in BALB/C mice that single intravenous injection of SAINT-MIX/ DNA complexes are safe and were free of detectable toxicity.

SAINT-MIX doses of 0, 1, 5, 15 en 75nmol with pCMV-EGFP were used. SAINT-MIX/ DNA ratio was 15nmol/ µg plasmid. Evaluation was carried out 2 and 14 days post injection. No effects were seen on food consumption and body weight. At 2 and 14 days post injection, no significant changes on blood chemistry, including creatinin, SGOT and SGPT were seen.

Pathology analysis of organs e.g. lung, liver, spleen, kidney, heart, intestine, ovary and brain revealed no abnormalities due to injection of the complexes. S. Audouy, G. Hosper et al (1) showed that SAINT-MIX/ DNA complexes are safe vehicles for *in vivo* gene-delivery.

The number of DNA copies applied in the maximal dose of this study is approximately 4 x10¹² copies, recalculated to a human body 6 x10¹⁴ DNA copies might be applied.

(1) S. Audouy, G. Hosper et al, *In vivo evaluation of the safety and toxicity of a new cationic liposome formulation for human gene therapy applications. AACR meeting, April 10-14 1999.*

(2) S. Audouy et al, *Serum as a modulator of lipoplex-mediated gene transfection: dependence of amphiphile, cell type and complex stability. J Gene Med 2000; 2: 465-476.*

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Latest findings on protocols, optimization, efficiencies and literature can be found on our web site.

- For optimal results, it is recommended to assay a range of concentrations (see example optimization).
- There is no need to wash away the SAINT-MIX/ DNA complex, because it constitutes **no** hazard to cells neither will it have a negative effect on transfection results.