

For research use only.

SAINT-RED siRNA/RNAi - Delivery System

- **Cat. No: SR-1003-01: 1mL package includes:** 1 vial with 1mL SAINT-RED and 1 vial with 12 mL Hepes Buffered Salt pH 7.4
- **Cat. No: SR-1003-02: 2mL package includes:** 1 vial with 2mL SAINT-RED and 2 vials with 12mL Hepes Buffered Salt pH 7.4
- **Cat. No: SR-1003-04: 4mL package includes:** 2 vials with 2mL SAINT-RED each and 3 vials with 15mL Hepes Buffered Salt pH 7.4

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1. Introduction

The transfection of siRNA 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 RNAi. Polyamines, polyimines and other polymers are the more common transfection reagents. 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.

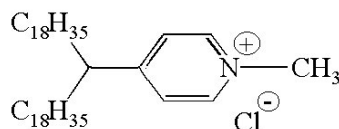
An important step forward is the development of amphiphiles as siRNA delivery systems.

SAINT, is a patented synthetic amphiphilic (non-liposomal) delivery system. This pyridinium surfactant enables non-toxic delivery of siRNA.

2. Product Description/Stability

Formulation: The SAINT-RED 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)methylpyridinium-chlorid) :



SAINT-RED consists of SAINT-18 and DOPE (Dioleoyl-phosphatidylethanolamine) dissolved in H₂O

Stability: SAINT-RED is supplied in H₂O, 'ready to use', and recommendedly stored at 4°–7°C. SAINT-RED 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-RED 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.

3. Quality Control

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

Cytotoxicity: SAINT-RED 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-RED.

Gene silencing has been achieved with minimal cellular damage. SAINT-RED has been used to transfect several animal and human cell-types/ lines, some with **more than 90% gene silencing**.

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-RED/ siRNA complex
- Purity and concentration of siRNA
- Cell culture (cell type , cell cycle, etc.)
- Verification of siRNA sequence

5.2. Recommended concentrations

In general, starting with a concentration of 15nmol SAINT-RED complexed with 50pmol siRNA 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-RED transfection pipetting scheme for several culture dishes

	6 well	12 well	24 well	48 well	96 well
SAINT-RED (µL)	20	10	5	2.5	1.25
HBS (µL)	80	40	20	10	5
siRNA * (pmol)	50	25	12.5	6	3
VOLUME (µL)	100	50	25	12	6
SAINT-RED (nmol)	15	7.5	3.75	1.875	0.937

* siRNA is diluted at 50 pmol per 100 µL HBS = 0.50 µM

You can optimize the siRNA / SAINT-RED ratio can for your application. Depending on the siRNA purity or GC content, a slightly different ratio might improve the transfection efficiency. An example for an optimization scheme in 96-well plates is shown beneath.

Example for an optimization in 96-well plates:

siRNA * (pmol)	VOLUME (μ L)	1 μ L	1.25 μ L	1.50 μ L	2.0 μ L
		SAINT-RED 5.25 μ L HBS	SAINT-RED 5 μ L HBS	SAINT-RED 4.75 μ L HBS	SAINT-RED 4.25 μ L HBS
1	2	A	E	I	M
3	6	B	F	J	N
4	8	C	G	K	O
5	10	D	H	L	P
SAINT-RED (nmol)		0.750	0.937	1.125	1.500

* siRNA is diluted at 50 pmol per 100 μ L HBS = 0.50 μ M

Example E:

1.25 μ L SAINT-RED (filled up with 5 μ L HBS) complexed with **3 pmol** of siRNA in 6 μ L HBS.

The endvolume of SAINT-RED/ siRNA complex is 12.25 μ L.

Fill this complex up to 65 μ L with medium of your choice and add it to the cells.

5.3. Handling instructions

Generally an amount of 3 pmol siRNA complexed with 1.25 μ L SAINT-RED gives a good transfection result in a 96-well.

5.3.1. Materials

Before use. SAINT-RED 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-RED/ siRNA complex for 96-well:

- Apply desired quantity of SAINT-RED and fill up to 6.25 μ L with HBS (included in package), HBSS or OptiMEM.
- Add 1 – 5 pmol siRNA to separate tube and fill up to 2 - 10 μ L with HBS (included in package), HBSS or OptiMEM. Leave to incubate during 5 minutes.
- Pipette the siRNA/ HBS solution into the SAINT-RED/ HBS solution and add this SAINT-RED/ siRNA complex to the cells.
- Fill up to 65 μ L with medium of your choice.

5.3.3. Transfection Protocol:

One day before transfection: Seed a **96-well** plate with approximately 10⁴ cells/ well. siRNA delivery should be performed when cells are subconfluent.

- Cells are to be washed once with HBS, HBSS or OptiMEM Some cell-lines showed better transfection results without washing
- Add the prepared SAINT-RED/ siRNA complex to each well and incubate 2 to 4 hours at 37°C
- Add 125 μ L medium, supplemented with serum, after 2 to 4 hours (leave SAINT-RED/ siRNA complex on the cells).
- After 24-96 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-RED for gene silencing is not influenced by serum.
- For optimal results, it is recommended to assay a range of concentrations (see example optimization).
- There is no need to wash away the SAINT-RED/ siRNA complex, because it constitutes no hazard to cells neither will it have a negative effect on transfection results.

6. References

The use of SAINT for the delivery of oligonucleotides is demonstrated by Shi *et al.* (1). Recently Stahmann *et al.* published their results using SAINT-RED for the down regulation of certain protein kinases in HUVEC cells (2).

(1) F. Shi *et al.*, *Efficient cationic lipid-mediated delivery of antisense oligonucleotides into eukaryotic cells: down-regulation of the corticotrophin-releasing factor receptor.* *Nucleic Acids Res* 2001; 29: 2079-2087.

(2) N. Stahmann, A. Woods, D. Carling, and R. Heller, *Thrombin Activates AMP-Activated Protein Kinase in Endothelial Cells via a Pathway Involving Ca²⁺/Calmodulin-Dependent Protein Kinase β .* *Mol. Cell. Biol.* Aug 2006, 5933-5945

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