

SAINT-PhD Protein high Delivery

- Cat. No: SP-2004-01: 1mL package includes:** 1 vial with 1mL SAINT-PhD and 1 vial with 12mL Hepes Buffered Salt pH 7.4
- Cat. No: SP-2004-02: 2mL package includes:** 1 vial with 2mL SAINT-PhD and 2 vials with 12mL Hepes Buffered Salt pH 7.4
- Cat. No: SP-2004-04: 4mL package includes:** 2 vials with 2mL SAINT-PhD each and 3 vials with 15mL Hepes Buffered Salt pH 7.4

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1. Introduction

The SAINT-PhD reagent is a superior protein delivery reagent, which can be used to deliver enzymes, antibodies, or other biologically functional proteins and peptides into living cells. Based on the successful SAINT cationic amphiphiles used for DNA transfections, Synvolux Therapeutics B.V. developed a novel formulation that contains the proprietary SAINT reagent mixed with other components optimized to the transfer of peptides and proteins.

A wide variety of mammalian cells can be used with SAINT-PhD, including B16-F10, COS-7, HEK293, CHO-K1, 3T3, HeLaS3, Jurkat, SKOV-3 and U373-MG. Also cell-lines, which are known to be difficult to deliver proteins are successfully used with this new SAINT-PhD reagent. Even primary endothelial cells (HUVEC) have shown great results.

Examples of proteins and enzymes that have been delivered to mammalian cells using SAINT-PhD include β -galactosidase, methyltransferase, and poly- or monoclonal antibodies. Additionally, fluorescently labeled proteins have been delivered with high efficiency.

Advantages using SAINT-PhD include:

- Ease of use
- Fast protocol, only 4 hours of incubation to deliver your protein
- No cytotoxicity

2. Storage and stability

It is recommended that SAINT-PhD is stored at 4-7 °C. Stability experiments have shown that SAINT-PhD stored at the recommended temperature is stable for at least one year after opening.

Furthermore, it has been found that protein delivery efficacy is not influenced by prolonged exposure (6 months) of the SAINT-PhD reagent to ambient temperature.

3. Quality control

Functionality: Protein delivery is performed on HeLa cells. Therefore, SAINT-PhD is tested with a labeled antibody.

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

4. SAINT-PhD protein delivery pipetting scheme for 6- and 24-well culture dishes

	6 well	24 well
SAINT-PhD (μ L)	80	20

Final Volume of protein dilution in HBS

	6 well	24 well
PROTEIN (μ g)	10	2
HBS (μ L)	120	30

Preparation of protein/SAINT-PhD complexes for 24-well plates

One day before protein delivery seed a 24-well plate with $\sim 10^4 - 10^5$ cells per well depending on the type of cells. For optimal protein delivery with SAINT-PhD, the cells should be confluent on the day of the experiment. Cell density could be optimized for each cell line used.

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

4.1. Standard protein delivery procedure by SAINT-PhD

Protein delivery in 24-well plate, assay after 4 hours, per well:

1. Dilute 2 μ g protein with the HBS (included in package) to a volume of 30 μ L. If the protein is too diluted do not use HBS buffer at all.
2. Pipette 20 μ L SAINT-PhD into the protein/HBS solution
3. After pipetting the SAINT-PhD the formulation may appear cloudy as complex formation occurs.
4. Fill up to 250 μ L with medium of your choice.
5. Aspirate the culture medium from the cells. Wash the cells once with HBS (*optional*).
6. Add the prepared SAINT-PhD/Protein complex (step 2) to each well and incubate 4 hours at 37 °C.
7. Incubate at 37°C in a 5% CO₂ incubator.
8. Perform your assay or if longer incubation time is required it might be necessary to fill up with extra medium.

4.2. Fast protein delivery procedure by SAINT-PhD

Protein delivery in 24-well plate, assay after 8-24 hours, per well:

1. Dilute 2 μ g protein with the HBS (included in package) to a volume of 30 μ L. If the protein is too diluted do not use HBS buffer at all.
2. Pipette 20 μ L SAINT-PhD into the protein/HBS solution.
3. After pipetting the SAINT-PhD the formulation may appear cloudy as complex formation occurs.
4. Add the prepared SAINT-PhD/Protein complex (step 2) directly to each well (drop-wise). Removal of growth medium is not necessary.
5. Swirl the plate gently to ensure an equally distribution over the entire plate surface.
6. Incubate at 37°C in a 5% CO₂ incubator.
7. Perform your assay after an appropriate incubation time.

5. Optimization

The protein / SAINT-PhD ratio may be optimized. Depending on the protein purity, molecular weight or isoelectric point, a different ratio might improve the protein delivery efficiency. Optimization is suggested for each new combination of cell line and protein. An example for an optimization scheme in 24-well plates is shown below.

Example for an optimization in 24-well plates:

PROTEIN (µg) *	SAINT-PhD			FINAL END VOLUME (µL) #
0.5	10µL	20µL	30µL	50
1	10µL	20µL	30µL	50
2	10µL	20µL	30µL	50
4	10µL	20µL	30µL	50

* Protein is diluted in HBS (included in the package)

Complex volume should be ~50µL including the SAINT-PhD

1. Start by using a fixed amount of SAINT-PhD (20µL).
2. Vary the amount of protein to be delivered (0.5-4µg).
3. The endvolume of SAINT-PhD/ Protein complex is 50µL.
4. Add this complex to the cells drop-wise (fast protocol) or fill up to 250µL with medium of your choice.
5. Incubate at 37°C in a 5% CO₂ incubator.
6. Analyze the cells after 4 hours or after a longer incubation period.
7. Do not remove the complex from the cells
8. The incubation time of the complex to the cells will influence the efficiency. Optimal delivery times should be determined empirically.
9. If further optimization is required, you can also vary the amount of SAINT-PhD.
10. With peptides or small proteins it can be useful using less SAINT-PhD, while large proteins require more of the reagent for efficient delivery.
11. Also the charge and hydrophobicity will influence the delivery.

Notes:

- a. *Before each protein delivery experiment new complex should be prepared and used immediately.*
- b. *For multiple delivery experiments, you can use a master mix required for multiple wells, but some proteins may cause aggregation when the complex is formed.*
- c. *The presence of serum has no significant effect on the protein delivery efficiency of SAINT-PhD. However, SAINT-PhD / protein complex should not be formed in the presence of serum.*
- d. *For optimal results, it is recommended to assay a range of concentrations of the protein or peptide used (see example optimization).*
- e. *As each protein has a different hydrophobicity and charge, the amount of SAINT-PhD may need to be optimized (see example optimization).*
- f. *There is no need to wash away the SAINT-PhD/ Protein complex, because it constitutes **no** hazard to cells neither will it have a negative effect on protein delivery results.*

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