

PepFection mRNA Transfection Reagent

(Cat #: T014)

Introduction:

The PepFection mRNA Transfection Reagent is a high efficiency mRNA transfection reagent, derived from Biodegradable non-toxic material and specifically designed for mRNA. This reagent can provide the robust transfection efficiency in a variety of commonly used and hard-to-transfect mammalian cells, such as adherent and suspension cells, as well as primary cells.

Advantages:

- ❖ Robust transfection efficiency and low cytotoxicity.
- ❖ Excellent for mRNA transfection.
- ❖ Compatible with serum and antibiotics in culture medium.

Table 1: Product Package & Storage

Cat #	Product Name	Volume	Storage
T014	PepFection mRNA Transfection Reagent	1 mL	2 ~ 8 °C, stable for up to 6 months
T014X		100mL	-20°C ,stable for up to 12 months

Important Guidelines for Transfection:

- (1) For maximum transfection efficiency, using serum-free medium (such as Opti-MEM® Reduced Serum Medium) to dilute mRNA and the PepFection mRNA Transfection Reagent is a must.
- (2) While the standard protocols for mRNA transfection being given below, optimization is often needed for maximal transfection efficiency.
- (3) It is unnecessary to wash cells and change medium after transfection.

Standard Protocol for mRNA Transfection of Adherent Cells

Step I . Cell Seeding:

Cells should be plated 18 to 24 hours prior to transfection so that the monolayer cell density reaches to 50 ~ 80% confluency at the time of transfection. Complete culture medium with serum and antibiotics is freshly added to each well 30 ~ 60 min before transfection.

Step II . mRNA Transfection Protocol

Use the following procedure to transfect mRNA into mammalian cells in a 6-well format. For other formats, please refer to **A Guideline for mRNA transfection (Table 2)**. All amounts and volumes are given on a per well basis. For each transfection sample, prepare complexes as follows:

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- a. Dilute 1ug mRNA in 125 μ L of serum-free Opti-MEM (or other medium without serum). Vortex to mix gently.
- b. Mix PepFection mRNA Transfection Reagent gently before use, then dilute 7 μ L of PepFection mRNA Transfection Reagent in 125 μ L of serum-free Opti-MEM.
- c. Add the diluted mRNA solution to the diluted PepFection mRNA Transfection Reagent all **(1:1 ratio)** at once. mix by pipetting up and down. Incubate for 15 minutes at room temperature to let transfection complex form well.
- d. Add the 250 μ L of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
- e. Incubate cells at 37°C in a CO₂ incubator for 24 to 72 hours. Then, to measure the gene silencing by qRT-PCR and Western Blotting respectively.

Table 2: A Guideline for mRNA transfection per cell culture vessel

Culture Vessel	Growth Medium (mL)	Amount of Plating Cells	Serum - Free Medium (μ L)	mRNA (ug)	PepFection mRNA Transfection Reagent(μ L)
	Volume used per well				
96-well	0.1	1 ~ 4 $\times 10^4$	2 \times 5	0.1	1
24-well	0.5	0.5 ~ 2 $\times 10^5$	2 \times 25	0.25	2
12-well	1.0	1 ~ 4 $\times 10^5$	2 \times 50	0.5	4
6-well	2.0	0.25 ~ 1 $\times 10^6$	2 \times 125	1	7