

Catalog No.: TS310

Product Name: LipoFexin

Size: 1ml

Description: **LipoFexin** transfection reagent will form complex with DNA or RNA, and this complex will efficiently enter into cells. It works well on a variety of adherent and suspension cell lines. It can be used for transfection of either DNA or RNA into eukaryotic cells in culture media with or without serum. **LipoFexin** is comparable to the Lipofectamine[®] 2000 transfection reagent at the molecular level and at the similar transfection efficiency. LipoFexin has the following Special Features:

- Superior transfection efficiency for a broad range of cell lines.
- No requirement of removal of serum from culture medium.
- No requirement for washing or changing of medium after transfection.
- Low cytotoxicity.

Storage: Store at 4°C.

Protocols

DNA Transfection:

The following protocol is for transfection of DNA into mammalian cells in a 6-well format. The amount and volume are given on a per well basis. Prepare the complex using a **1:2 to 1:3 ratio** of DNA (**µg**) to LipoFexin (**µl**) for most cell lines. Do the transfection at a high cell density for high efficiency, high expression level, and to minimize cytotoxicity. Optimization may be necessary for different cell lines.

1. **For Adherent cells:** One day before transfection, plate $0.5-2 \times 10^5$ cells in 2ml of growth medium without antibiotics so that cells will be 60-90% confluent at the time of transfection. **For Suspension cells:** Just prior to preparing complexes, plate $4-8 \times 10^5$ cells in 2ml of growth medium without antibiotics.
2. For each transfection, prepare the **Transfection Complexes** as follows:
 - a. Mix the LipoFexin before use; prepare two sterile microtubes and mark them as **Tube A** and **Tube B**.
 - b. In **Tube A**, pipette the desired amount DNA per well (e.g., 2.0µg/well) in 200µl of Opti-MEM medium without serum and antibiotics (or other similar medium without serum and antibiotics). Mix well but gently by pipetting up and down a few times.
 - c. In **Tube B**, pipette the appropriate amount of LipoFexin (e.g., 6µl/well) in 200µl of the Opti-MEM medium without serum and antibiotics. Mix well but gently by pipetting up and down a few times.
 - d. Transfer the DNA in **Tube A** to the **Tube B** (The total volume is 400µl). Mix gently and incubate at room temperature for 20 minutes to form the **DNA-LipoFexin complex** (solution may appear cloudy). **Note:** The formed complexes are stable for 6 hours at room temperature.
3. Add the 400µl complex drop-wise to the well containing the cells. Mix gently by rocking the plate back and forth a few times.
4. Incubate the cells at 37°C in a CO₂ incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 6 hours for some sensitive cells, but not necessary for most cell lines.
5. **For generating stable cell lines:** Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection, and add selective reagent the following day.

Optimizing DNA Transfection: To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as the ratio of DNA to LipoFexin. Make sure that cells are greater than 90% confluent and vary the **DNA(μg): LipoFexin (μl)** ratios in the range of **1:1 to 1:5** to find the optimal/best ratio for particular cell lines under the current transfection condition.

RNA Transfection:

Use the following procedure to transfect RNA into mammalian cells in a 24-well format. For other formats, see section of **Scaling Up or Down Transfections**. All amounts and volumes are given on a per well basis. Use this procedure as a starting point; optimize transfections as described in **Optimizing RNA Transfection**.

1. One day before transfection, plate cells in 500μl of growth medium without antibiotics such that they will be 30-50% confluent at the time of transfection. **Note:** Transfecting cells at a lower density will allow a longer interval between transfection and assay time, and minimizes the loss of cell viability due to overgrowth of cells.
2. For each transfection, prepare the **Oligomer-LipoFexin Complexes** as follows:
 - a. In **Tube A**, dilute 20pmol Stealth™ RNAi or siRNA oligomer in 50μl Opti-MEM medium without serum and antibiotics (final concentration of RNA when added to the cells is 33nM). Mix gently.
 - b. In **Tube B**, dilute 1μl of LipoFexin in 50μl Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature. **Note:** Mix the LipoFexin well but gently before use.
 - c. After the 5-minute incubation, combine the diluted oligomer with the diluted LipoFexin. Mix gently and incubate for another 20 minutes at room temperature (the complex solution may appear cloudy).
3. Add the Oligomer-LipoFexin Complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth a few times.
4. Incubate the cells at 37°C in a CO₂ incubator for 24-96 hours until you are ready to assay, such as for gene knockdown. Medium may be changed after 4-6 hours.

Optimizing RNA Transfection: To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA to LipoFexin ratio in the range of 10-50pmol RNA to 0.5-1.5 μl LipoFexin for a 24- well format transfection, and proportionally adjust the RNA to LipoFexin ration for other formats. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions.

Scaling Up or Down Transfection

To transfect cells in different tissue culture formats, vary the amounts of the transfection reagent, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the table below. With automated, high-throughput systems, a transfection complex solution volume of 50μl is recommended for transfections in 96-well plates. **Note:** You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly add cells at twice the cell density as in the basic protocol. Cells will adhere as usual in the presence of complexes.

Culture vessel	Surface area (cm ²)	Medium volume	Transfection complex volume	DNA transfection		RNA transfection	
				DNA	LipoFexin	RNA	LipoFexin
96-well	0.3	100μl	2 × 25μl	0.2μg	0.5μl	5pmol	0.25μl
24-well	2	500μl	2 × 50μl	0.8μg	2.0μl	20pmol	1.0μl
12-well	4	1ml	2 × 100μl	1.6μg	4.0μl	40pmol	2.0μl
6-well	10	2ml	2 × 250μl	4.0μg	10μl	100pmol	5.0μl
6-cm dish	20	5ml	2 × 500μl	8.0μg	20μl	200pmol	10μl
10-cm dish	60	15ml	2 × 1.5ml	24μg	60μl	600pmol	30μl