LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: TS310

Product Name: **LipoFexin**

Size: 1ml

Description: **LipoFexin** is a lipid-based transfection reagent that forms complex with DNA or RNA, and transports the complex into cells. It works 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** has been compared to work at the similar way and efficiency as the Lipofectamine® 2000 transfection reagent. 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 24-well format. For other formats, see the section of **Scaling Up or Down Transfection**. All amounts and volumes are given on a per well basis. Prepare complexes 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 levels, and to minimize cytotoxicity. Optimization may be necessary for different cell lines (see **Optimizing DNA Transfection**).

1. **For Adherent cells:** One day before transfection, plate 0.5-2 x 10^5 cells in 500µl of growth medium without antibiotics so that cells will be 70-90% confluent at the time of transfection. **For Suspension cells:** Just prior to preparing complexes, plate 4-8 x 10^5 cells in 500µl of growth medium without antibiotics.
2. For each transfection, prepare the **Transfection Complexes** as follows:
    a. Prepare two new/sterile microtubes, and mark them as Tube A and Tube B.
    b. In **Tube A**, dilute the desired amount DNA per well (e.g., 0.8µg/well) in 50µl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix well gently.
    c. In **Tube B**, dilute the appropriate amount of LipoFexin (e.g., 2µl/well) in 50µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature. **Note:** Mix the LipoFexin gently before use.
    d. After the 5 minute incubation, combine the diluted DNA in Tube A with the diluted LipoFexin in Tube B (The total volume is 100µl). Mix gently and incubate another 20 minutes at room temperature (solution may appear cloudy). **Note:** The formed complexes are stable for 6 hours at room temperature.
3. Add the 100µl 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 CO_2 incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.
5. **For stable cell lines:** Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium (if desired) 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 1:0.5 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® I Reduced Serum Medium without serum (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 CO_2 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

This product is for research use only.