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EZ-XpressFect

Catalog Number	Size
EZXF90-1	EZ-XpressFect Transfection Reagent: 1.0 ml 10X EZ Buffer: 1 x 2.0 ml
EZXF90-2	EZ-XpressFect Transfection Reagent: 2 x 1.0 ml 10X EZ Buffer: 2 x 2.0 ml
EZXF90-5	EZ-XpressFect Transfection Reagent: 5 x 1.0 ml 10X EZ Buffer: 5 x 2.0 ml

Description:

EZ-XpressFect combines a simple and fast protocol with outstanding transfection efficiency and low toxicity. Transfections can be performed efficiently using a fixed DNA-lipid ratio, rendering time-consuming optimization obsolete. Since the new protocol of EZ-XpressFect takes only three days to complete, it enables two consecutive tests to be performed within one week. EZ-XpressFect shows no serum inhibition and is the reason for sensitive cells.

Application: Transfection of nucleic acids into mammalian cells

Formulation: Cationic lipids with co-lipids in water

Assays: Up to 1500 (24-well) or up to 400 (6-well) with 1 ml reagent

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like EZ-XpressFect change their size distribution after long storage at +4°C, which can have slight adverse effects on the transfection efficiency. This effect can be reversed by a freeze-thaw cycle. It is recommended to perform a freeze-thaw cycle before first use, and subsequently monthly to yield optimal results.

Note: This product is for research use only. Not for use in human or animal diagnostics, therapeutics, or clinical applications.

General Guidelines

State of Cells

Cells to be transfected should be well proliferating and healthy. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for

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transfection experiments. Microbial contamination with mycoplasma or fungi can drastically alter transfection results and must be excluded.

Quality of the Nucleic Acid

To achieve optimum transfection results the DNA used should be of maximum possible purity. Endotoxins and other contaminants can drastically reduce transfection efficiency.

Transfection Protocol

Notes

Tolerance to transfection reagents may vary, sometime significantly, depending on cell types or cell lines. In the following protocol, cells are transfected in two wells using two different volumes of DNA-lipid complex (lipoplex).

The evaluation of this assay serves as the basis for deciding which of the two lipoplex volumes is ideal for the transfected cells. This volume must be determined empirically for each cell type.

If the lipoplex volume suitable for the cells in question is already known, only this volume need be used. The pipetting charts are given in the **Values for Known Suitable Lipoplex Volumes** section.

The following instructions apply to wells with 1 cm^2 growth surface (equivalent to a single well of a 48-well plate). A conversion chart for other well formats is given in the **Conversion to Other Well Formats** section.

Reagent Preparation

1. EZ Buffer – Make 1X EZ Buffer from the provided 10X EZ Buffer. To do this, mix 1 part 10X EZ Buffer with 9 parts of sterile water suitable for cell culture under sterile conditions.
2. Prior to transfection, bring 1X EZ Buffer and EZ-XpressFect Transfection Reagent to room temperature and vortex shortly. Bring the DNA solution to room temperature and mix gently.

Cell Preparation

1. Prepare 500 µl of cell suspension at a concentration of 4 – 8 x 10^5 cells/ml in complete cell culture medium.
2. Fill two wells of a 48-well plate (well 1 and well 2) with 250 µl of cell suspension each.

Lipoplex Preparation

1. Place 75 µl 1X EZ Buffer in the test tube (ideally polypropylene).
2. Pipette 2.0 µl EZ-XpressFect Transfection Reagent into the 1X EZ Buffer and mix the solution by gently pipetting up and down once.
3. Add 2.0 µg DNA by pipetting and mix as before.
   Note: Mix gently, shearing forces damage the lipoplex and reduce transfection efficiency.
4. Incubate for 15 minutes at room temperature.

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Transfection

1. Add the lipoplex solution to the wells of cell suspension as follows:

   Well 1: 25 µl of lipoplex

   Well 2: 50 µl of lipoplex

2. Mix the solutions in both wells by gently pipetting up and down once.
3. Incubate the wells under normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).

Evaluation

Evaluation generally takes place 24 – 72 hours later. The best results/highest protein production are generally obtained after 48 hours. The optimum time is determined on the basis of the properties of the cells' expression product and promoter activity.

Conversion to Other Wells Formats

Format	Area	Cell suspension	1X EZ Buffer	EZ-XpressFect Transfection Reagent	DNA	Lipoplex volume	
						Well 1	Well 2
96–well	0.3 cm^2	2× 100 µl	30 µl	0.6 µl	0.6 µg	10 µl	20 µl
48–well	1.0 cm^2	2× 250 µl	75 µl	2.0 µl	2.0 µg	25 µl	50 µl
24–well	1.9 cm^2	2× 500 µl	150 µl	3.8 µl	3.8 µg	50 µl	100 µl
12–well	3.6 cm^2	2× 900 µl	300 µl	7.2 µl	7.2 µg	100 µl	200 µl
6–well	9.0 cm^2	2× 2.2 ml	600 µl	18 µl	18 µg	200 µl	400 µl
60 mm dish	22 cm^2	2× 5.5 ml	1.5 ml	44 µl	44 µg	500 µl	1.0 ml
100 mm dish	60 cm^2	2× 15 ml	4.5 ml	120 µl	120 µg	1.5 ml	3.0 ml

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Values for Known Suitable Lipoplex Volumes

	Lipoplex volume 1			Lipoplex volume 2		
Format	**1X EZ Buffer**	**EZ-XpressFect Transfection Reagent**	**DNA**	**1X EZ Buffer**	**EZ-XpressFect Transfection Reagent**	**DNA**
96–well	10 µl	0.2 µl	0.2 µg	20 µl	0.4 µl	0.4 µg
48–well	25 µl	0.7 µl	0.7 µg	50 µl	1.3 µl	1.3 µg
24–well	50 µl	1.3 µl	1.3 µg	100 µl	2.5 µl	2.5 µg
12–well	100 µl	2.5 µl	2.5 µg	200 µl	5.0 µl	5.0 µg
6–well	200 µl	6 µl	6 µg	400 µl	12 µl	12 µg
60 mm dish	500 µl	15 µl	15 µg	1.0 ml	29 µl	29 µg
100 mm dish	1.5 ml	42 µl	42 µg	3.0 ml	78 µl	78 µg

Additional Notes

The culture medium should be replaced when exhausted, particularly when rapidly growing cells are used.

When highly sensitive cells are used, replacement of the medium approximately 6 – 8 hours after seeding may be useful.

If lower cell densities are desired, the volume of cell suspension can be reduced by up to 30%.

When growth is underway (6 – 8 hours) culture medium should be added.