Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

RhB-XpressFect

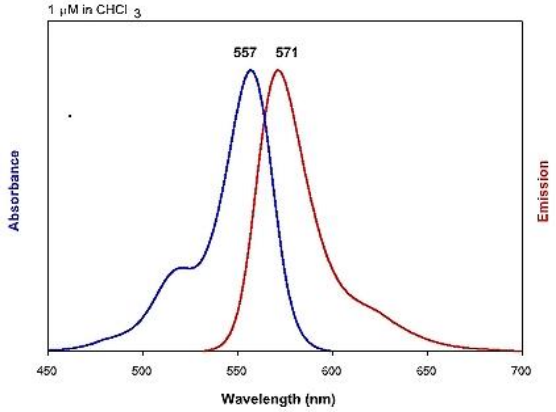
Catalog Number	Size
RhBXF50-0.5	0.5 ml
RhBXF50-1	1.0 ml

Description:

RhB-XpressFect is a fluorescence-labeled reagent for transfection of eukaryotic cells with DNA. RhB-XpressFect is rhodamine B labeled to enable the transfection process to be tracked and its success evaluated by means of fluorescence microscopy or FACS.

Application: Transfection of eukaryotic cells with DNA; visual tracking of lipoplex during and after the transfection process.

Formulation: Cationic lipids with co-lipids in water; covalently bonded rhodamine B label (Ex_{max} = 557 nm, Em_{max} = 571 nm)



Assays: 1 ml reagent up to 2500 (24-well) or 625 (6-well)

Shipping: Shipped at room temperature.

Storage: +4°C

Stability: See label for expiration date.

Formulations of liposomes like RhB-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.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

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 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

In transfection of eukaryotic cells, the cell nucleus is the intended site of action of the DNA. Among many impeding factors, the two principal barriers are the cell membrane and nuclear membrane. The transfection reagent enables the DNA to pass the cell membrane by combining with the DNA to form a complex (lipoplex) which is then actively introduced into the cytoplasm by means of endocytosis. Division of the transfected cells is critical to this process because the lipoplex is not in itself able to pass the nuclear membrane; the DNA can only pass into the cell nucleus during cell division when the nuclear membrane opens.

The maximum transfection efficiency rises as the cell division rate increases during treatment of the cells with the lipoplex. The cell division rate of adherent cells is primarily determined by their cell density (cells/cm^2). The higher the cell density, the higher the proliferation rate and the higher the maximum transfection efficiency.

Microscopy calls for cell densities far lower than those achieved in optimum proliferation of adherent cells since lower cell densities enable the morphology of individual cells to be observed more closely.

The RhB-XpressFect protocol was developed for the purpose of microscopy analysis. The maximum achievable transfection efficiency is constrained by the relatively low cell proliferation rate, given the need for low cell density. This protocol gives recommended initial values for cell concentration, plasmid amount and DNA:lipid ration (µg: µl). Cell density should be optimized for the cell type and requirements of the assay in question to achieve successful transfection results. See the Protocol Optimization section for more details.

The following instructions refer to one well with 1 cm^2 proliferation area (one well of a 48-well plate). For other well sizes, see Conversion to Other Well Formats section.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Cell Preparation

1. On the **first day** of the assay, prepare 250 µl of cell suspension with a concentration of 0.5-1.0 x 10^5 cells/ml in complete culture medium and add the suspension to one well of a 48-well plate.
2. Incubate the suspension for 24 hours at 37°C in an atmosphere containing CO_2.

Lipoplex Preparation and Addition

1. On the **second day**, bring the RhB-XpressFect and the DNA solution to room temperature and briefly vortex prior to lipoplex formation.
2. Prepare the following solutions in sterile vessels appropriate for cell culture (preferably polypropylene). **Always introduce the medium first** to prevent the reagent and DNA solutions from coming into direct contact with the vessel surface:
   DNA Solution: 0.05 – 0.2 µg DNA (initial value: 0.1 µg) into 15 µl serum and antibiotic-free medium
   Lipid Solution: 0.2 – 1.5 µl RhB-XpressFect (initial value: 0.5 µl) into 15 µl serum and antibiotic-free medium
   The volumes of DNA and lipid given are sufficient for a DNA:lipid ratio (µg/µl) of 1:4 – 1:6.5. The recommended initial vale is 1:5 µg/µl.
3. Add the DNA solution to the lipid solution and mix gently by pipetting up and down once. Incubate the solution for 15 – 20 minutes at room temperature.
   Note: The DNA solution must be added to the lipid solution. DO NOT add the lipid solution to the DNA solution.
4. Add 30 µl of the lipoplex solution to the incubated cells in from the Cell Preparation step. Agitate gently to mix (high shearing forces can damage the DNA-lipid complex). Incubate at 37°C in an atmosphere containing CO_2.
5. Five (5) hours after adding the lipoplex, replace the medium with fresh complete culture medium.

Evaluation

The rhodamine label enables the transfection process to be visualized, and lipoplex localization and uptake to be tracked. In addition, RhB-XpressFect can be used in combination with a suitably fluorescence-labelled DNA to deliver information on the localization of the genetic material introduced, the genetic product and the lipoplex.

The time of evaluation should be selected with respect to the object of evaluation. As a basic principle, microscopic observation (e.g. live imaging) is possible from the point where the lipoplex is added; however, care should be taken to replace the medium (as stated in the protocol) 5 hours after lipoplex addition. Replacing the medium before this point will result in significantly reduced transfection rates.

Expressed protein amounts frequently peak after 48 hours. The optimum time is determined by the characteristics of the cell type and expression product as well as promoter activity. To optimize image quality for fluorescence or transmitted-light microscopy, the medium can be replace by 1X PBS in the final evaluation.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Conversion to Other Wells Formats

Format	Area	Cell suspension	Medium for lipoplex formation	RhB-XpressFect Transfection Reagent	DNA	Lipoplex volume
96–well	0.3 cm^2	100 µl	2 x 10 µl	0.1 – 0.65 µl (0.25 µl)	0.025-0.1 µg (0.05 µg)	20 µl
48–well	1.0 cm^2	250 µl	2 x 15 µl	0.2 – 1.3 µl (0.5 µl)	0.05-0.2 µg (0.1 µg)	30 µl
24–well	1.9 cm^2	500 µl	2 x 30 µl	0.4 - 2.6 µl (1.0 µl)	0.1-0.4 µg (0.2 µg)	60 µl
12–well	3.6 cm^2	1000 µl	2 x 50 µl	0.8 – 5.2 µl (2.1 µl)	0.2-0.8 µg (0.4 µg)	100 µl
6–well	9.0 cm^2	2000 µl	2 x 100 µl	1.6 – 10.4 µl (4.2)	0.4-1.6 µg (0.8 µg)	200 µl
60 mm dish	22 cm^2	5000 µl	2 x 250 µl	3.9 – 26.0 µl (10.4)	1.0-4.0 µg (2.0 µg)	500 µl
100 mm dish	60 cm^2	15000 µl	2 x 750 µl	10 - 65 µl (26 µl)	2.5-10 µg (5.0 µg)	1500 µl

The chart gives the recommended initial reagent volume and DNA amount for each format in parenthesis.

Protocol Optimization

Optimum Cell Density

The potential level of transfection efficiency is strongly affected by cell density at the time of adding the lipoplex (see Notes under Transfection Protocol). Therefore, the first parameter to be optimized should be the cell concentration of the cell suspension seeded on the first day.

The goal I to achieve the best possible balance between observability (best at low cell density) and transfection rate (best at high cell density). The prepared cell concentration should be as high as possible while still allowing evaluation to be carried out at the desired time.

For most cell types, preparation of a cell suspension at a concentration of 0.5-1.0 x 10^5 cells/ml leads to full confluency after 2 – 3 days. This recommended initial concentration should be adjusted depending on the requirements of the experiment.

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Optimization Protocol

In the following optimization protocol, cells are transfected in nine vessels (wells or dishes) with three DNA amounts (I, II and III) and three different DNA:lipid rations (1:4, 1:5, 1:6.5 µg/µl). To achieve this, three different lipoplex solutions are prepared at the given DNA:lipid ratios and three wells are filled with three different volumes of solution.

The following example shows the nine resulting lipoplex compositions for a 48-well format optimization:

	DNA:Lipid Ratio in µg:µl ➡		
DNA amount ⬇	1:4, 0.05 µg	1:5, 0.05 µg	1:6.5, 0.05 µg
	1:4, 0.1 µg	1:5, 0.1 µg	1:6.5, 0.1 µg
	1:4, 0.2 µg	1:5, 0.2 µg	1:6.5, 0.2 µg

The optimum parameters for DNA amount and DNA:lipid ratio apply only to the specific combination of the selected cell type and cell density.

Seeding the cells

On the first day of the assay, prepare a cell suspension using the concentration determined for the cell type in question and distribute among nine culture vessels. The volume of cell suspension is given in the Conversion to Other Wells Formats chart on the previous page.

Incubate the suspension for 24 hours under normal conditions for the cell used (e.g. 37°C in CO_2 atmosphere).

Solution Preparation

Label four reaction vessels (ideally made of polypropylene) “DNA solution”, “Lipid 1:4”, “Lipid 1:5”, and “Lipid 1:6.5”.

Add the DNA solution and three lipid solutions to the corresponding vessels. The following chart shows the volumes of medium, reagent, and DNA amounts for each culture format.

The following example applies to wells of a 48-well plate each with 1 cm^2 growth area:

DNA solutions: 90 µl serum and antibiotic-free medium + 1.2 µg DNA

Lipid Solutions:

1:4 30 µl serum and antibiotic-free medium + 4.8 µl RhB-XpressFect

1:5 30 µl serum and antibiotic-free medium + 6.0 µl RhB-XpressFect

1:6.5 30 µl serum and antibiotic-free medium + 7.8 µl RhB-XpressFect

Express Biotech International
4650 Wedgewood Blvd., STE 103
Frederick, MD 21703 USA

Tel: +001.301.228.2444
Fax: +001.301.560.6570
Email: xpressbio@xpressbio.com

Reference Chart for Other Wells Formats

Format	Growth area (cm^2)	Medium for DNA solution (µl)	DNA amount (µg)	Medium for lipid solutions (µl)	Lipid volume (µl)		
					1:4	1:5	1:6.5
96–well	0.3	60	0.6	3× 20	2.4	3.0	3.9
48–well	1.0	90	1.2	3× 30	4.8	6.0	7.8
24–well	1.9	180	2.4	3× 60	9.6	12.0	15.6
12–well	3.6	300	4.8	3× 100	19.2	24.0	31.2
6–well	9.0	600	9.6	3× 200	38.4	48.0	62.4
60 mm dish	22	1500	24	3× 500	96	120	156
100 mm dish	60	4500	60	3× 1500	240	300	390

Lipoplex Formation

Add 30 µl of DNA solution to each of the three lipid solutions, 1:4, 1:5, and 1:6.5, and mix gently by pipetting up and down once. **Note: The DNA solution must be added to the RhB-XpressFect solution. Do not add the RhB-XpressFect solution to the DNA solution.**

Incubate the solution for 15 – 20 minutes at room temperature.

Adding the lipoplex

Pipette the lipoplex solution into the nine culture vessels of proliferating cells. Add three different volumes of each of the three lipoplex solutions 1:4, 1:5, and 1:6.5 as follows:

Format	Lipoplex volume (µl)		
	V_1	V_2	V_3
96–well	5	10	20
48–well	7.5	15	30
24–well	15	30	60
12–well	25	50	100
6–well	50	100	200
60 mm dish	125	250	500
100 mm dish	375	750	1500

Incubate under the normal conditions for the cell line used (e.g. 37°C in atmosphere containing CO_2).