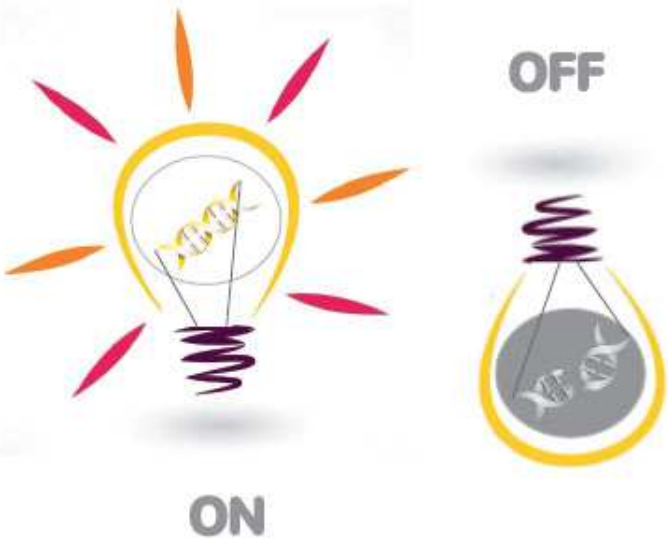
Lullaby™ siRNA Transfection reagent

INSTRUCTION MANUAL



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Lullaby® siRNA transfection reagent

Instruction Manual

Lullaby® siRNA transfection reagent is a very efficient reagent specifically designed for all siRNA applications

List of **Lullaby® siRNA transfection reagent** Kits

Catalog Number	Description	Volume (µL)	Number of transfections[1] / 24 well plates	Number of transfections[1] / 96 well plates
LL70500	Lullaby® siRNA transfection reagent	500	250	1000
LL71000	Lullaby® siRNA transfection reagent	1000	500	2000
LL73000	Lullaby® siRNA transfection reagent	3x1000	1500	6000

[1] Number of transfection given for a concentration of 10 nM siRNA.

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (order@ozbiosciences.com). For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).

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1. Technology

1.1. Description

Congratulations on your purchase of the **Lullaby® siRNA transfection reagent**!

RNA interference allows gene silencing in mammalian cells. Specifically designed short RNA duplexes (siRNA) interact very selectively with their mRNA target triggering its sequence specific degradation and protein synthesis inhibition. Efficient transfection of these siRNA is a critical step for effective gene silencing. **Lullaby® siRNA transfection reagent** was specially designed to achieve this goal. The cationic lipids formulation protects siRNA from extracellular degradation, transport them across cell membranes and efficiently release the siRNA into cells thanks to a triggered endosomal escape mechanism. In this way, ***Lullaby®*** formulation gives reliable higher gene silencing efficiencies in numerous cell types than any other transfection reagent. Moreover, highly efficient transfection and gene silencing are achievable even with low doses of siRNA.

Principal **Lullaby® siRNA transfection reagent** advantages:

1. Exceptional siRNA delivery efficiency and gene silencing (>90%)
2. Suitable for all siRNA applications (co-transfection, endogenous gene silencing)
3. Effective gene silencing at multiple siRNA concentrations including low doses (<10 nM) – minimize the risk of off-target effects
4. Rapid and Straightforward procedure (3-step)
5. Powerful across a broad spectrum of cells and confluency (from 20 to 90%)
6. Reliable and reproducible gene knockdown results
7. Serum compatible & Non toxic
8. Highly adapted to high-throughput siRNA screening

1.2. Kit Contents

Kit contents vary according to their size:

- 1 tube containing 0.5 mL of **Lullaby® siRNA transfection reagent** good for up to 1000 assays.
- 1 tube containing 1 mL of **Lullaby® siRNA transfection reagent** good for up to 2000 assays.
- 3 tubes containing 1 mL each of **Lullaby® siRNA transfection reagent** good for up to 6000 assays.

Stability and Storage

Storage +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. **Lullaby® siRNA transfection reagent** kits are stable for at least 12 months at the recommended storage temperature.

Shipping condition Room Temperature

2. Applications

2.1. siRNA Mediated Gene Silencing

RNA interference is a powerful technique to shut down genes expression in cells and organisms. This silencing effect constitutes a very helpful tool to study gene's function and a promising approach for new therapeutic treatments. Short RNA duplexes (siRNA: small interfering RNA, shRNA: small hairpin RNA and dsRNA: double strand RNA) are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein production. **Lullaby® siRNA transfection reagent** introduces the siRNA duplexes in a variety of cells with a very high efficiency leading to exceptional knockdown effects with low doses of siRNA. **Lullaby ®** is suitable for small RNA such as siRNA, shRNA and dsRNA.

2.2. Cell Types and Targets

Lullaby® siRNA transfection reagent is applicable on numerous cell types and multiple targets. This reagent has been tested on a variety of cells and OZ Biosciences is maintaining an updated list of cells successfully tested that is available on the website: www.ozbiosciences.com. If a particular cell type is not listed, this does not imply that **Lullaby® siRNA transfection reagent** is not going to work. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

Cell Line	*Cell Type*	*Source*
A549	Non-small cell lung carcinoma	Human
CHO-K1	Epithelial-like (Ovary)	Hamster
COS-1, COS-7	Fibroblast (Kidney)	Green Monkey
H441	papillary adenocarcinoma (Lung)	Human
HEK-293	Transformed Embryonic Kidney	Human
HeLa	Cervical Epithelial Carcinoma	Human
M-1	Epithelial (Kidney)	Mouse
MDCK	Normal -Kidney	Canine
MIAPaCa-2	Epithelial (Pancreas)	Human
NIH3T3	Fibroblast	Mouse
U87	Glioblastoma	Human
Vero 10A1	Epithelial (Kidney)	Monkey

Targeted reporter genes (co-transfection and stably transfected cells): GFP, Luciferase, Lac Z...
Targeted endogenous genes: GAPDH, Lamin, kinases...

3. Protocol

3.1. General Considerations

The instructions given below represent model protocols that were applied successfully on several cells. Our R&D team has extensively tested and optimized **Lullaby® reagent** in order to provide you with the simplest, straightforward and efficient procedure. Therefore, we recommend you to start by following our general protocol as guidelines to obtain good data quickly and if necessary, we advise you to optimize the transfection parameters in order to achieve the best effects. Optimal conditions do vary from cell to cell and are highly dependent upon the siRNA sequence and the gene targeted. Consequently, the amount, concentration and ratio of the individual components (siRNA and reagents), the time course and the number of cells may have to be adjusted to get the best results. Several optimization protocols are available in the Appendix.

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency.
- **siRNA** should be as pure as possible, use high quality siRNA (PAGE purified and desalted). Endotoxin levels must be very low since they hamper transfection efficiencies. We suggest mixing quickly the siRNA solution and Lullaby®reagent in serum free medium to avoid any degradation or surface adsorption.
- **Antibiotics**. The absence of antibiotics from the media has been reported to improve transfection efficiency. We did not observe a significant effect of the presence or absence of antibiotics with Lullaby®.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the siRNA and transfection reagent solutions. RNAse-free materials and buffer are favorable to handle siRNA.

3.2. General Protocol

It is recommended to seed or plate the cells the day prior transfection, however cells can also be prepared few hours before the transfection or the reverse transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Best results are achieved if cells are at least 50-70 % confluent at the time of transfection (see the suggested cell number in the table 1).

Lullaby® siRNA transfection reagent is suitable for transfection and reverse transfection

The addition of the siRNA / **Lullaby®** mixtures prepared in serum-free medium will result in the dilution of serum, antibiotics or other additives of your standard culture medium. A medium change is not required after transfection for most cell types; but, it may be needed for cells sensitive to serum/supplement concentration.

Table 1: Recommended number of cells to seed.

Culture vessel	Number of adherent cells	Number of suspension cells	Cell overlay volume
96-well	$6 – 12 \times 10^3$	$4 – 8 \times 10^4$	100 µL
24-well	$4 – 8 \times 10^4$	$25 - 50 \times 10^4$	400 µL
12-well	$8 – 15 \times 10^4$	$5 – 10 \times 10^5$	900 µL
6-well	$2 – 4 \times 10^5$	$1 – 2 \times 10^6$	1800 µL
60 mm dish	$5 – 10 \times 10^5$	$2 – 5 \times 10^6$	3800 µL
90 – 100 mm dish	$10 – 20 \times 10^5$	$4 – 10 \times 10^6$	7800 µL

3.3. siRNA Transfection Procedure

The protocol is very straightforward. Please refer to the tables below for specific amount of the respective compounds and transfection volume. For instance:

- Use 2 µL of **Lullaby®** reagent for 10 nM siRNA final concentration in a 24-well plate (volume 0.5 mL)
- Use 8 µL of **Lullaby®** reagent for 10 nM siRNA final concentration in a 6-well plate (volume 2 mL)

1) Plate the cells the day before transfection or just before transfection (suspension cells) in your appropriate tissue culture dish and volume of culture medium as suggested in Table 1.

2) Dilute the siRNA in 50 or 100 µL of culture medium **without** serum and supplement (such as DMEM) (see Table 2 for siRNA dilution procedure). The siRNA optimal concentration required to achieve the best gene silencing effect depends highly on the cells, target and siRNA sequence; consequently, we suggest to first test a range of siRNA concentration from 1 to 50nM.

Table 2: Suggested dilution procedure and amount of siRNA to test:

Culture vessel	96-well		24-well		12-well		6-well	
Dilution serum-free medium	50 µL		50 µL		50 µL		100 µL	
	Amount of siRNA (1 µM stock)*							
Final siRNA concentration	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**
1 nM	0.2	2.7	0.5	6.75	1	13.5	2	27
2 nM	0.4	5.4	1	13.5	2	27	4	54
5 nM	1	13.5	2.5	33.75	5	67.5	10	135
10 nM	2	27	5	67.5	10	135	20	270
25 nM	5	67.5	12.5	168.75	25	337.5	50	675
50 nM	10	135	25	337.5	50	675	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

3) Dilute **Lullaby®** reagent to 50 or 100 µL with culture medium **without** serum and supplement (such as DMEM) (see Table 3 for dilution procedure). The **Lullaby®** reagent (store at +4°C) should have an ambient temperature and be gently vortexed prior to use. It is important to add first the serum free medium to the tube and then add carefully the **Lullaby®** reagent **directly into the serum free medium** without touching any plastic surface. As a starting point you can use the amount of Lullaby® indicated in Table 3, however we recommend to start your first experiment by testing several doses of **Lullaby®** reagent with one concentration of siRNA as detailed in Table 4 in order to achieve the best effects.

Table 3: Recommended amount of **Lullaby®** reagent per nM of siRNA used:

Culture vessel	96-well	24-well	12-well	6-well
Final transfection volume	200 µL	500 µL	1 mL	2 mL
	Amount of reagent			
Final siRNA concentration				
1 to 5 nM	0.5 µL	1 µL	2 µL	4 µL
10 nM	0.5 µL	2 µL	4 µL	8 µL
25 nM	1 µL	3 µL	6 µL	10 µL
≥ 50 nM	1 µL	4 µL	8 µL	14 µL

4) Add the 50 or 100 µL of siRNA-diluted solution into the **Lullaby**® reagent diluted solution tube and mix immediately 4–5 times by pipetting up and down.

   Note: We recommend respecting the order of addition; add the siRNA solution into the Lullaby solution.
5) Incubate the mixture (siRNA / **Lullaby**® reagent) 20 minutes at room temperature.
6) Add the 100 or 200 µL of complexes drop by drop directly onto the cells. The final transfection volumes per well (culture medium + siRNA / **Lullaby**® reagent mixture) are shown in the Table 3.

   Note: For some cells, serum-free condition for the first 3 hours of incubation might lead to better gene silencing. However, in most assays, siRNA delivery has been realized in culture medium with serum.
7) Cultivate the cells under standard conditions until evaluation of the gene silencing. Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection. We recommend 24h and 72h for RNA and protein knockdown analyses, respectively.

   Note: optionally, culture medium can be changed 24 or 48 hours post-transfection.

3.4. siRNA Reverse Transfection Procedure

The reverse transfection procedure is identical to the transfection procedure (section 3.3) except that siRNA / **Lullaby**®complexes are first added directly in an empty cell culture dish (steps 2 to 5) and then cells prepared in culture medium containing serum (step 1) are added in the well/dish onto the preformed complexes (steps 6 to 7). Cell number suggested in Table 1 have to be adjusted because for reverse transfection they are not seeded 24h prior transfection and thus 1.5 to 2 fold more cells should be used.

4. Appendix

4.1. Critical Parameter for best performance

1) Cell culture conditions: Best results are achieved when cells are 50–70 % confluent at the time of the transfection. If necessary, you can wash the culture medium containing the transfection mixture after 8-24 hours and replace it by fresh medium.
2) siRNA concentration. We often observed good siRNA effects at very low concentrations from 0.1 to 5nM. However, the efficiency may depend on the cell line, the target (half life, expression level...) and the siRNA used. Consequently, we suggest you to start by testing a range of siRNA concentration in order to obtain the best experimental conditions.
3) Time course. The gene silencing time course depends on the amount/concentration of siRNA used. Indeed, with high quantity of siRNA, very efficient gene expression knockdown can be observed at earliest time point such as 16 or 24 hours. In contrast, with low siRNA concentration gene silencing require longer incubation such as 48 or 72 hours. At first, we suggest to monitor gene silencing at 72h post-transfection.

4.2. Optimization Protocol

In order to get the best out of **Lullaby**® **siRNA transfection reagent**, several parameters can be optimized:

- Ratio of **Lullaby**® reagent to siRNA
- siRNA dose used, which strongly depends on the efficiency and specificity of your siRNA
- Cell type, cell density and incubation time

OZ Biosciences team has investigated numerous factors during the course of the R&D program. Based on our experience, we recommend that you optimize one parameter at a time and start from the experimental procedures described above. Then, the following optimization can be accomplished:

1) Start by optimizing the ratio **Lullaby**® / siRNA. To this end, use a fixed amount of siRNA and vary the amount of **Lullaby**® as detailed in the Table 4. The reagents can be pre-diluted in deionized water and aliquots of the resulting dilutions are incubated with siRNA. Diluted **Lullaby**®solution has to be freshly prepared.

Table 4: Recommended amount of **Lullaby®** per nM of siRNA used:

Culture vessel	96-well	24-well	12-well	6-well
Final transfection volume	200 µL	500 µL	1 mL	2 mL
		Amount of Lullaby®		
Final siRNA concentration				
5 nM	0.25 - 0.5 – 1 – 1.5µL	0.5 - 1 – 2 - 3µL	1 – 2 – 3 - 4µL	2 – 4 – 6 - 8µL
10 nM	0.25 - 0.5 – 1 – 1.5µL	1 – 2 – 3 - 4µL	2 – 4 – 6 - 8µL	4 – 8 – 12 - 16µL
25 nM	0.5 - 1 – 2 - 3µL	1.5 – 3 – 4 - 6µL	3 – 6 – 9 - 12µL	7 - 10 – 15 - 20µL
50 nM	0.5 - 1 – 2 - 3µL	2 - 4 - 6 – 8µL	4 – 8 – 12 - 16µL	10 - 14 – 18 - 22µL

2) Thereby, optimize the siRNA dose with the fixed ratio of **Lullaby®** / siRNA that has been previously optimized (Table 4).

3) After having identified the optimal quantity of **Lullaby®** reagent and siRNA, you could pursue the process by optimizing the cell number (density) and time course of your experiment.

4.3. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Lullaby® reagent / siRNA ratio.** Although our reagent has been extensively optimized and fixed volumes are provided, optimization may be required. Optimize the Lullaby/siRNA ratio as described in the optimization protocol. Briefly, use a fixed amount of siRNA (10 or 20nM) and vary the amount of **Lullaby®** reagent from 2 times less up to three times more than the suggested amount detailed in the Table 3 2- **siRNA amount.** Use different concentration of siRNA with the recommended or optimized Lullaby/siRNA ratio. 3- **Cell density.** A non-optimal cell density at the time of siRNA delivery can lead to insufficient uptake. The optimal confluency should range from 50 to 70% but most favorable cell density may vary according to the cell type. 4- **siRNA quality.** Use high quality siRNA (PAGE purified and desalted). Employ RNAse-free materials and check for siRNA integrity on acrylamide gel. Ensure siRNA is not denatured. 100mM NaCl, 50mM Tris pH7.5 RNAse-free buffer can be used for siRNA instead of water. 5- **Mycoplasma contamination.** Mycoplasma contamination alters transfection efficiency. 6- **Cell condition.** Cells that have been in culture for a long time may become resistant to transfection. Use freshly thawed cells that have been passaged at least once.
Cellular toxicity	1- **Concentration of Lullaby® siRNA transfection reagent / siRNA.** Decrease the amount of siRNA / reagent complexes added to the cells. 2- **Incubation time.** Reduce the incubation time of complexes with the cells. Transfection medium can be replaced by fresh medium after 4h. 3- **siRNA quality.** Use high quality siRNA as impurities can lead to cell death. 4- **Key gene silencing.** If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the siRNA this can lead to cell death 5- **Unhealthy cells.** Check cells for contamination, use new batch of cells, ensure culture medium condition (pH, type of medium used, contamination etc)
No or weak gene silencing effect	1- **siRNA design.** The design of an efficient siRNA is a crucial step. Ensure to use a validated siRNA sequence. If a validated siRNA cannot be used, assay your sequence in an easy to transfect cell line (if possible) in order to validate it (HeLa cells for example). 2- **siRNA concentration.** Use higher amount of siRNA. 3- **Incubation time.** Perform a time-course experiment to set up the optimal incubation time since gene silencing is dependent on the gene expression and the protein turnover rate.

	4- **Incorrect medium used for preparing Lullaby/siRNA complexes**. It is critical that serum-free medium or buffer (HBS, PBS) are used during the preparation of the Lullaby/siRNA complexes. 5- **Old Lullaby/siRNA complexes**. The Lullaby/siRNA complexes must be freshly prepared every time. Complexes prepared and store for longer than 1 hour can be aggregated. 6- **Improper storage. Lullaby®** siRNA transfection reagent is very stable at 4°C but high temperature and/or excessive freeze/thaw cycles may cause lost of reagent activity.

Our dedicated and specialized technical support team will be pleased to answer any of your requests and to help you with your transfection experiments at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

4.4. Quality Controls

To assure the performance of each lot of **Lullaby® siRNA transfection reagent** produced, we qualify each component using rigorous standards. The following assays are conducted *in vitro* to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
Purity	Silica Gel TLC assays. Every compound shall have a single spots.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Gene silencing efficacies in GFP stably transfected HeLa and MDCK cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

5. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

Purchaser Notification

Limited License

The purchase of **Lullaby® siRNA transfection reagent** grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of **Lullaby® siRNA transfection reagent**. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

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Product Use Limitations

The **Lullaby® siRNA transfection reagent** is developed, designed, intended, and sold for research use only. It is not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

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