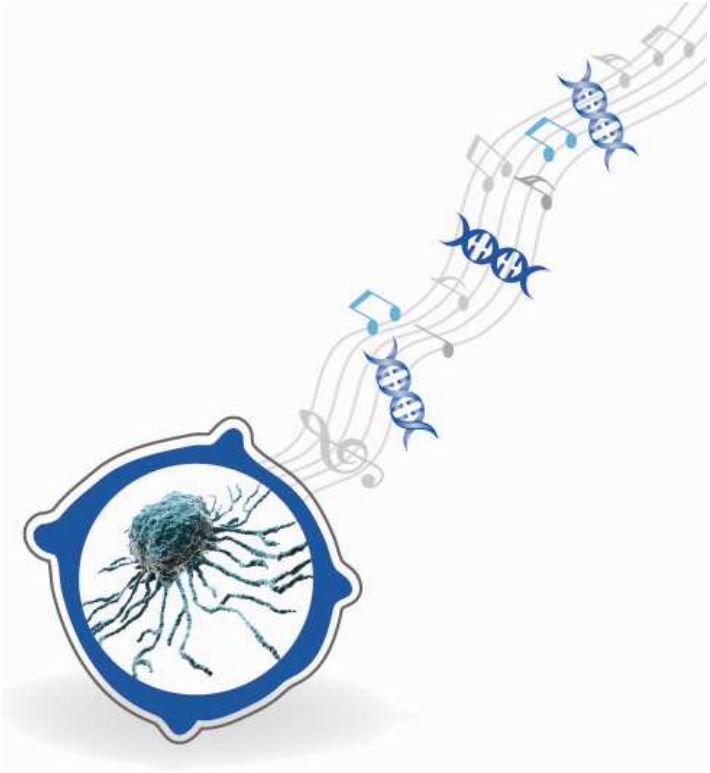
Lullaby® Stem siRNA Transfection Reagent

INSTRUCTION MANUAL

Stem Cell-specific transfection reagent



OZBIOSCIENCES
The art of delivery systems
www.ozbiosciences.com

www.ozbiosciences.com

Lullaby® Stem

Instruction Manual

Lullaby® Stem was especially developed to transfect small RNAs (siRNA, miRNA) into multipotent and embryonic stem cells.

List of Lullaby® Stem siRNA transfection reagent kits

Catalog Number	Description	Volume (µL)	Number of transfection in a 24-well plate*	Number of transfections[1] in a 96-well plate*
LS20500	Lullaby® Stem	500	250	1000
LS21000	Lullaby® Stem	1 000	500	2000

* Number of transfection is given for a concentration of 10 nM siRNA.

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

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - France
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

Table of contents

1. Technology

1.1. Description

For a better understanding of this protocol, small non-coding RNA molecules (siRNA, miRNA, piRNA...) will be refered as siRNA, please adapt your experimental conditions to the molecule you work with. ***Lullaby® Stem*** *is suitable for all kinds of small RNA.*
For transfection of shRNA or pre-miR plasmids in embryonic and multipotent stem cells, we highly recommend the use of OZ Biosciences' DreamFect Stem.

RNA interference in stem cells allows crucial studies for addressing issues in stem cells biology and for developing gene therapy strategies. Specifically designed, small non-coding RNA molecules interact very selectively with their mRNA targets triggering its sequence specific degradation, translational repression and finally protein synthesis inhibition. Efficient transfection of these molecules is a critical step for effective gene silencing. **Lullaby® Stem** siRNA transfection reagent was specially designed to achieve this goal in embryonic and multipotent stem cells. The cationic lipids formulation protects siRNA from extracellular degradation, allows its transport across stem cell membranes and efficiently release the small RNA into the cytoplasm thanks to a triggered endosomal escape mechanism. In this way, **Lullaby® Stem** formulation gives reliable higher gene silencing efficiencies and low toxicity in numerous stem cell types. Moreover, highly efficient transfection and gene silencing are achievable even with low doses of siRNA. Consequently, stem cells keep their undifferentiated stage and their capacities to differentiate.

Lullaby® Stem main advantages in comparison to standard procedures:

1. High transfection efficiency for embryonic and multipotent stem cells
2. Minimized toxicity due to reagent biodegradability and low siRNA amount required
3. Does not affect phenotype and differentiation potential
4. Serum Compatible
5. Simple, Ready-to-use and rapid (no specific buffer)

1.2 Kit Contents

OZ Biosciences offers two sizes of Lullaby® Stem reagent:

- 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.

Stability and Storage

Storage: Upon reception and for long-term use, store the reagent at +4°C.
Lullaby® Stem is stable for at least one year at the recommended storage temperature.

Shipping condition: Room Temperature.

2. Applications

2.1. Application Areas

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 is a promising approach for new therapeutic treatments. Small RNAs are extremely selective by interacting and inducing the degradation of their specific mRNA targets and thereby inhibit the resulting protein synthesis. **Lullaby® Stem** transfection reagent introduces the small RNA into embryonic and multipotent stem cells with a very high efficiency leading to exceptional knockdown effects with low doses of siRNA. **Lullaby® Stem** has been developed for small RNA such as siRNA and miRNA.

2.2. Cell Types

Lullaby® Stem reagent is suitable for a variety of stem cells such as multipotent stem cells like Mesenchymal Stem Cells (MSC), Adipose-derived Stem Cells (AdSC), Amniotic Fluid Stem Cells (AFSC), embryonic stem cells (H9) as well as Neural Stem Cells. An updated list of cells tested with **Lullaby® Stem** is available at: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com, so we can update this list and give you all the support you need.

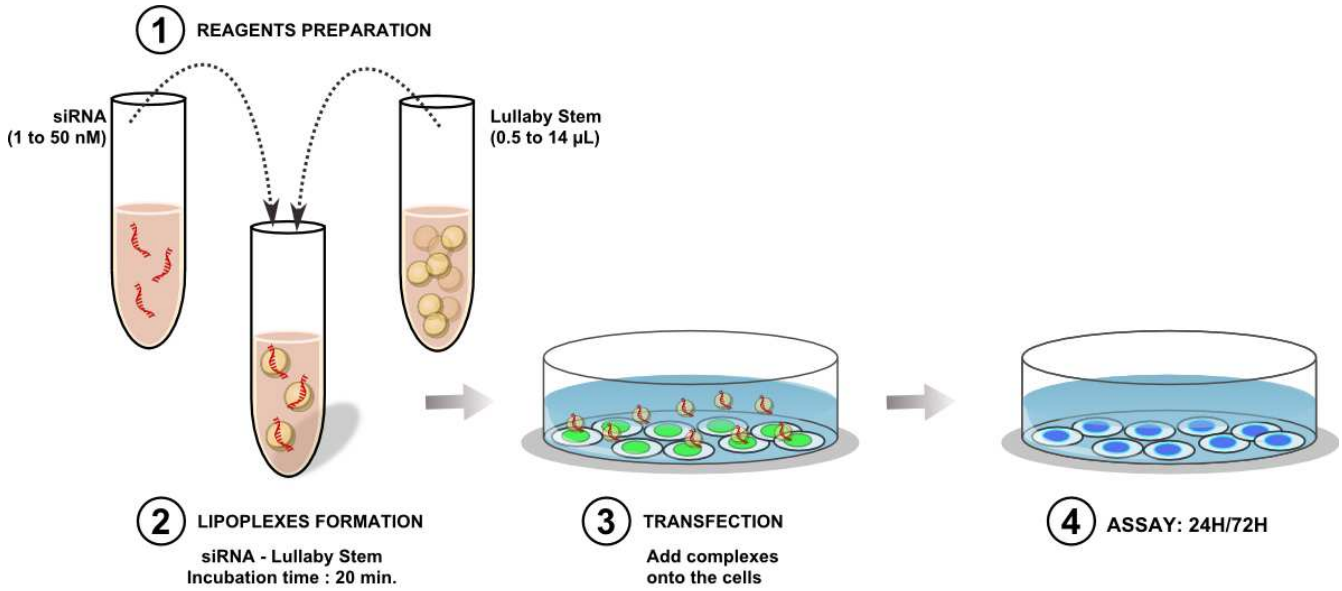
3. Protocols

3.1. General Considerations

The instructions given below were successfully applied on several stem cells. Our R&D team has extensively tested and optimized Lullaby® Stem reagent in order to provide you with the simplest, straightforward and efficient procedure. However, since conditions may vary from cell to cell and are highly dependent upon the siRNA sequence and the gene targeted, you might need to optimize the transfection parameters in order to achieve the best results. Consequently, amount, concentration and ratio of the individual components (siRNA and reagents), time course and number of cells might have to be adjusted to get the best results (please see 3.3 for optimization protocols).

- **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® Stem reagent in serum free medium to avoid any degradation or surface adsorption.
- **Antibiotics**. 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® Stem.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the siRNA and transfection reagent solutions. RNAse-free materials and buffer are preferred to handle siRNA.

3.2. Rapid Protocol



- Seed the cells the day prior transfection. The cells should be at 50-70% confluent at the time of transfection (40% for embryonic stem cells).
- Use cells with a low number of passages.
- Lullaby® Stem solutions should be at room temperature and be gently vortexed prior to use.

Table 1: Suggested dilution procedure and siRNA amount to test

Cell culture dish	96 well plate		24 well plate		12 well plate		6 well plate	
Dilution volume	50 µL		50 µL		50 µL		100 µL	
Amount of siRNA (1µM 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	57
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

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

2. **Lullaby® Stem solution**. Dilute **Lullaby® Stem** reagent to 50 or 100 µL with culture medium (such as DMEM) WITHOUT serum and supplement (see Table 2 for dilution procedure). 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.

3. Add the siRNA solution to the **Lullaby® Stem** solution, mix gently by pipetting up and down 4-5 times. Incubate the mixture for 20 min at room temperature.
   The diluted solutions should be combined within 5 minutes.

4. Add the mixture dropwise to the cells growing in complete medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

5. Incubate the cells at 37°C in CO2 incubator under standard conditions.

6. *Optional*: Cell medium could be changed with fresh complete medium after 4 to 6h incubation.

7. Evaluate your transgene expression after 24 to 72 h following transfection.

Table 2: Suggested amount of Lullaby® Stem reagent per nM of siRNA used

Cell culture dish	96 well plate	24 well plate	12 well plate	6 well plate
Dilution volume	50 µL	50 µL	50 µL	100 µL
Amount of Lullaby Stem				
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

3.3. Optimization Protocol

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

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

Based on our experience, we recommend you to 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® Stem** / siRNA. To this end, use a fixed amount of siRNA and vary the amount of **Lullaby® Stem** as detailed in the Table 3. The reagents can be pre-diluted in culture medium WITHOUT serum and supplement (such as DMEM) and aliquots of the resulting dilutions are incubated with siRNA. Diluted **Lullaby® Stem** solution has to be freshly prepared.

Table 3: Recommended amount of **Lullaby®** Stem 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 Stem				
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) Thereafter, optimize the siRNA dose with the fixed ratio of **Lullaby® Stem** / siRNA that has been previously optimized (Table 4).

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

4. Appendix

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

4.1 Quality Controls

To assure the performance of each lot of Lullaby® Stem, we qualify each component using rigorous standards procedures. The following *in vitro* assays are conducted 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 spot.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Gene silencing efficacies on GFP-stably transduced MSC cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **Optimization of Lullaby® Stem / nucleic acid ratio.** See section 3.3. 2- **siRNA amount.** Use different concentration of siRNA with the recommended or optimized (above) Lullaby® Stem / siRNA ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency for multipotent stem cells should range from 50 to 70% (true confluency, corresponding to 90% visual confluency) but most favorable cell density may vary according to the cell type; preferably mid-log growth phase. 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. TRIS buffer (100mM NaCl, 50mM Tris pH7.5) can be used for siRNA instead of water. Employ nuclease-free materials. 5- **Mycoplasma contamination.** Mycoplasma contamination alters transfection efficiency. 6- **Cell condition.** 1) Cells should have the less passage as possible (< P5). 2) Cells that have been in culture for a long time (> 8 weeks) may become resistant to transfection. 3) Cells should be healthy and assay during their exponential growing phase. 4) The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **Medium used for preparing siRNA / Lullaby® Stem complexes**. It is critical that serum-free medium (DMEM, Opti-MEM) or buffer (HBS, PBS) are used during the preparation of the complexes. 8- **Cell culture medium composition.** 1) For some cells, transfection efficiency can be increased without serum or under reduced serum condition. Thus, transfect these cells in serum-free medium during the first 4h of incubation. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, gene targeted, etc. The transfection efficiency can be monitored after 24 – 96h by analyzing the gene product. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours.

	10- **Old transfection reagent / siRNA complexes.** The Lullaby® Stem / siRNA complexes must be freshly prepared each time to avoid aggregation. 11- **Silencing detection assay**. Ensure that your post-transfection assay is properly set up and includes a positive control (*i.e* fluorescent siRNA). 12- **Transfection reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials 2- **Key gene silencing.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a control or scrambled siRNA. 3- **siRNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 5- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.

5. Related Products

Description
Stem cells dedicated reagents
DreamFect Stem Stem (*for all nucleic acids)*
RmesFect Stem *(for mRNA)*
Cellular Senescence kit
MAGNETOFECTION TECHNOLOGY
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*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)*
***In vivo* Magnetofection**
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
Lullaby (*siRNA transfection reagent)*
RmesFect *(Transfection reagent for mRNA)*

Do not hesitate to contact us for all complementary information and visit our website to stay inform on our last breakthrough technologies and updates.

contact@ozbiosciences.com / www.ozbiosciences.com

Purchaser Notification

Limited License for *Lullaby® Stem*

The purchase of *Lullaby® Stem* Reagents 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 and transduction of nucleic acids and virus 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® Stem* Reagents. 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.

Buyers may end this License at any time by returning all *Lullaby® Stem* Reagents material and documentation to OZ Biosciences, or by destroying *Lullaby® Stem* Reagents components. Purchasers are advised to contact OZ Biosciences with the notification that *Lullaby® Stem* Reagents kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of *Lullaby® Stem* Reagents research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations for *Lullaby® Stem*

Lullaby® Stem Reagents and all of its components are developed, designed, intended, and sold for research use only. They are 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:

OZ Biosciences SAS
Director of Business Development
Parc Scientifique et Technologique de Luminy
163, avenue de Luminy, zone entreprise, case 922
13288 Marseille Cedex 9, France
Tel: +33 (0)4.86.94.85.16
Fax: +33 (0)4. 86.94.85.15
E-mail: business@ozbiosciences.com

www.ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

www.ozbiosciences.com

Follow us!