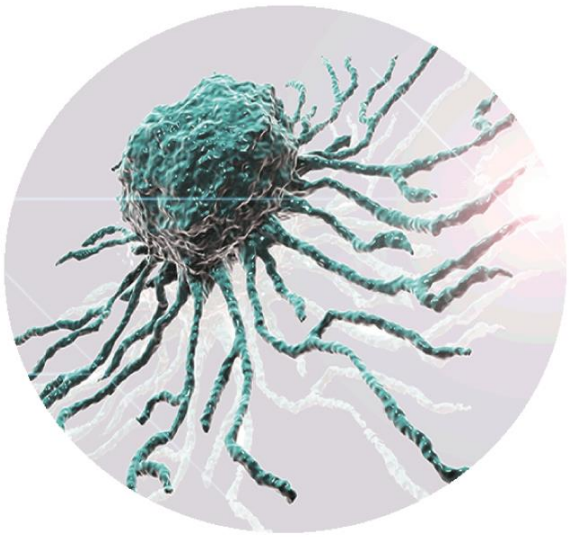


Stem Cell Applications

Lullaby® Stem siRNA

Stem cell-specific transfection reagent

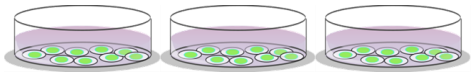
Protocol

OZBIOSCIENCES
The art of delivery systems

Lullaby® Stem Quick Protocol

To find your ideal silencing conditions with Lullaby® Stem, we suggest to test increasing doses of siRNA (or miRNA): from 10 to 50nM per well.

1 **Seed cells to be at 50-70% confluent the day of transfection**



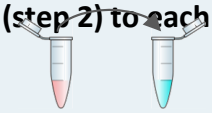
2 **Prepare 3 tubes of siRNA (with different amounts of nucleic acids)***

96 well plate	24 well plate	6 well plate
10 nM/25nM/50nM in 50µL serum-free medium or buffer*	10 nM/25nM/50nM in 50µL serum-free medium or buffer*	10 nM/25nM/50nM in 100µL serum-free medium or buffer*

3 **Prepare 3 tubes of Lullaby® Stem (with different amounts of reagent)***

96 well plate	24 well plate	6 well plate
0.5µL/1µL/1µL in 50µL of serum-free medium or buffer*	2µL/3µL/4µL in 50µL of serum-free medium or buffer*	8µL/10µL/14µL in 100µL of serum-free medium or buffer*

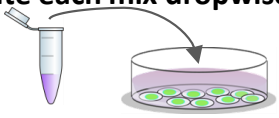
4 **Mix each tube of siRNA (step 2) to each tube of Lullaby® Stem (step 3)***



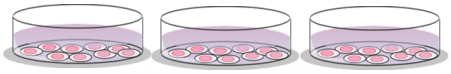
96 well plate			24 well plate			6 well plate		
siRNA		*Lullaby®*	*siRNA*		*Lullaby®*	*siRNA*		*Lullaby®*
10nM	+	0.5µL	10nM	+	2µL	10nM	+	8µL
25nM	+	1µL	25nM	+	3µL	25nM	+	10µL
50nM	+	1µL	50nM	+	4µL	50nM	+	14µL

5 **Incubate 20 min at room temperature**

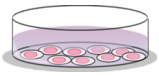
6 **Distribute each mix dropwise onto the cells**



7 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene silencing**



8 **Choose the best ratio siRNA:Lullaby® Stem**



These conditions might require some further optimizations depending on your cells, siRNA, target, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ 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 10 to 50nM.
- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ For the preparation of Lullaby Stem. 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.
- ✓ Medium or buffer without serum & supplement must be used for the siRNA/Lullaby Stem complexes preparation. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ We recommend respecting the order of addition of reagents: add the siRNA solution into the Lullaby Stem solution.
- ✓ Dilute the reagent with deionized water for doses less than 1µL,

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	LS20500: 500µL of Lullaby Stem LS21000: 1mL of Lullaby Stem
Shipping conditions	Room Temperature
Storage conditions	Store the Lullaby Stem transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	Lullaby Stem is the ideal siRNA transfection reagent for gene silencing in stem cells.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

- 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.
- See the suggested cell number in the Table 1.

Culture vessel	Number of adherent cells
96-well	6 – 12 x 10^3
24-well	4 – 8 x 10^4
6-well	2 – 4 x 10^5

Table 1: Recommended number of cells to seed

2. siRNA/Lullaby Stem complexes preparation

The siRNA and Lullaby Stem solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to Table 2) of culture medium without serum and antibiotics.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

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

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

b. *Lullaby Stem preparation*. Dilute 0.5 to 14 µL of Lullaby Stem in 50 or 100 µL (refer to Table 3) of culture medium without serum and antibiotics.

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Final siRNA concentration	*Amount of Lullaby (µl)*		
10 nM	0.5	2	8
20nM	1	3	10
≥ 50 nM	1	4	14

Table 3: Recommended amount of Lullaby Stem per nM of siRNA used

c. Add the siRNA solution onto the Lullaby Stem reagent and mix gently by carefully pipetting up and down.

d. Incubate the mixture for 15-20 minutes at room temperature. Do not vortex or centrifuge!

3. Transfection

a. Add the complexes dropwise onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis.

NOTE: Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection.

Protocol | siRNA in suspension cells

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. siRNA/Lullaby Stem complexes preparation

The siRNA and Lullaby solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (refer to Table 2) of culture medium without serum and antibiotics.

b. *Lullaby Stem preparation*. Dilute 0.5 to 14 µL of Lullaby in 50 or 100 µL (see Table 3) of culture medium without serum and antibiotics.

c. Add the siRNA solution onto the Lullaby reagent and mix gently by carefully pipetting up and down.

d. incubate the mixture for 15-20 minutes at room temperature. Do not vortex or centrifuge!

3. Transfection

a. While the complexes are incubating, prepare your cells in serum-free medium (or serum-containing medium) and transfer the appropriate volume to the culture dish according to Table 4. In 24-well plates for instance plate 2x10^5 suspension cells just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

Culture vessel	Number of suspension cells
96-well	4 – 8 x 10^4
24-well	2.5 - 5 x 10^5
6-well	1 – 2 x 10^6

Table 4: Recommended number of suspension cells to seed

b. Next, add the complexes directly onto the cells dropwise and all over the well. Important: gently mix complexes with the cells by pipetting the culture medium up and down (3-4 times to disrupt potential cell clumps and to ensure contact of the complexes with cells

c. Incubate 3 to 6 h (4h is commonly used) in serum-free medium at 37°C under 5% CO2.

d. If transfections are performed in serum free medium, add serum to adjust its concentration.

e. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis.

NOTE: Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection

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

1. Start by optimizing the siRNA dose with the fixed ratio of **Lullaby® Stem** / siRNA that has been previously optimized (refer to Table 5).

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Final siRNA concentration	*Amount of Lullaby (µl)*		
5 nM	0.25 – 0.5 – 1 – 1.5	0.5 – 1 – 2 - 3	2 – 4 – 6 - 8
10 nM	0.25 – 0.5 – 1 – 1.5	1 – 2 – 3 - 4	4 – 8 – 12 - 16
20nM	0.5 – 1 – 2 - 3	1.5 – 3 – 4 - 6	7 – 10 – 15 - 20
≥ 50 nM	0.5 – 1 – 2 - 3	2 – 4 – 6 - 8	10 – 14 – 18 - 22

Table 5: Recommended amount of Lullaby® per nM of siRNA used

2. Thereafter, optimize 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 4. The reagents can be pre-diluted in culture medium (such as DMEM) without serum and supplement and aliquots of the resulting dilutions are incubated with siRNA. Diluted **Lullaby® Stem** solution has to be freshly prepared.

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.

Additional products for your transfection experiments:

- **DreamFect Stem** for stem cells transfection with DNA
- **RmesFect Stem** for stem cells transfection with mRNA

Purchaser Notification

Limited License
The purchase of the Lullaby Stem kit grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in this protocol). 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 the Lullaby Stem kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all Lullaby Stem kit reagents and documentation to OZ Biosciences, or by destroying all Lullaby Stem components. Purchasers are advised to contact OZ Biosciences with the notification that a Lullaby Stem 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 the Lullaby Stem kit 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
Lullaby Stem kit 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.

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