

Lullaby® Stem– Results

Stem cells are widely investigated due to their regenerative potential. In many cases, it would be useful to enhance or silence some of their features through gene delivery strategies to harness their full potential cell/gene-based therapy. Because stem cells behavior, physiology and properties are quite distinctive to other cells, OZ Biosciences has developed a specific transfection reagent for small RNA application. Lullaby® Stem enables siRNA and miRNA transfection of multipotent and embryonic stem cells with high efficiency and very low toxicity. Its lipid-specific formulation protects small RNA from extracellular degradation, transports them across cell membranes and efficiently releases them into stem cell cytoplasm.

Lullaby™ Stem Benefits:

1. High transfection efficiency of multipotent and embryonic stem cells
2. Minimized toxicity due to reagent biodegradability and low siRNA/miRNA amount required
3. Reliable and reproducible gene knockdown results
4. Serum & Antibiotics Compatible
5. Simple, Ready-to-use and rapid

High gene silencing efficiency mediated by Lullaby™ Stem / siRNA complexes

Lullaby Stem allows high silencing efficiency on stem cells such as human Mesenchymal Stem Cells (MSC; Fig. 1, Fig. 3) and Amniotic Fluid Stem Cells (AFSC; Fig. 2, Fig. 3)

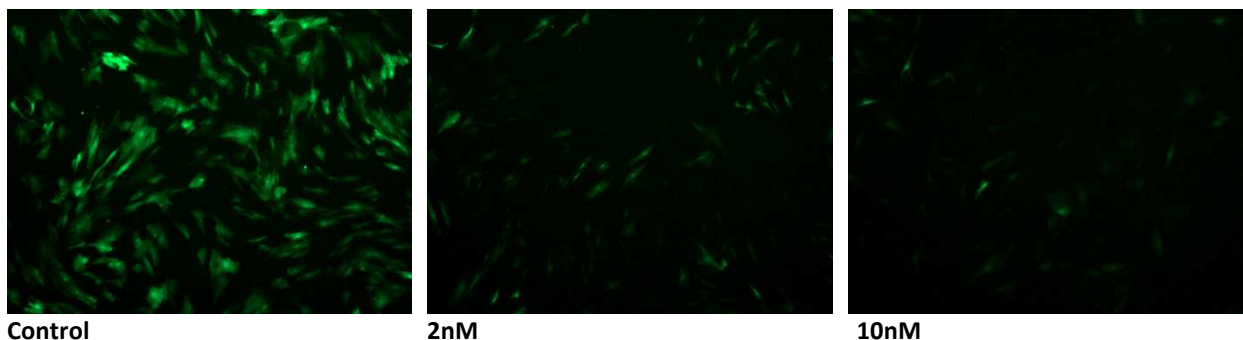


Figure 1: Human MSC stably expressing the GFP protein were treated with **Lullaby® Stem** transfection reagent and siRNA (targeting GFP). Cells were seeded in 6 well-plates in 2 mL of complete medium and complexes were prepared with 8µL of Lullaby Stem and 0 (Control well), 2 or 10 nM of siRNA. GFP expression was monitored 72h post transfection by fluorescent microscopy (x200).

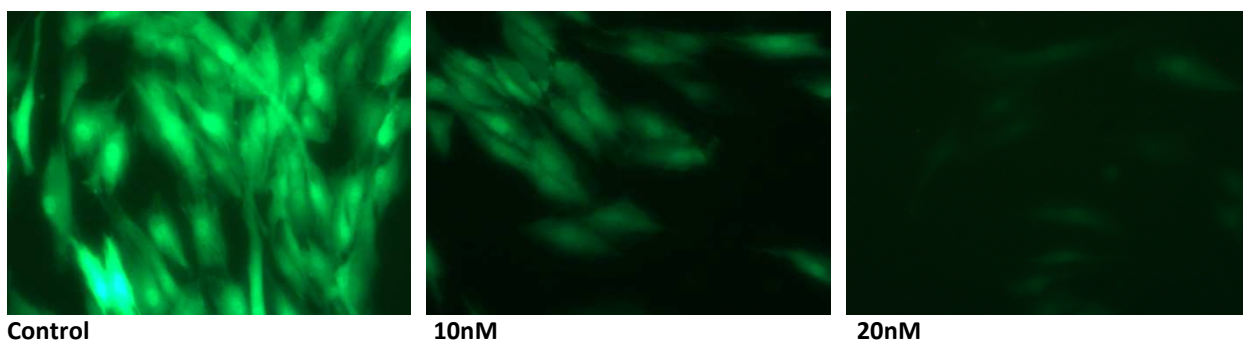


Figure 2: Representative pictures of GFP-stably transduced human AFSC 48h after treatment with **Lullaby® Stem** transfection reagent and increasing doses of siRNA targeting GFP (x400).

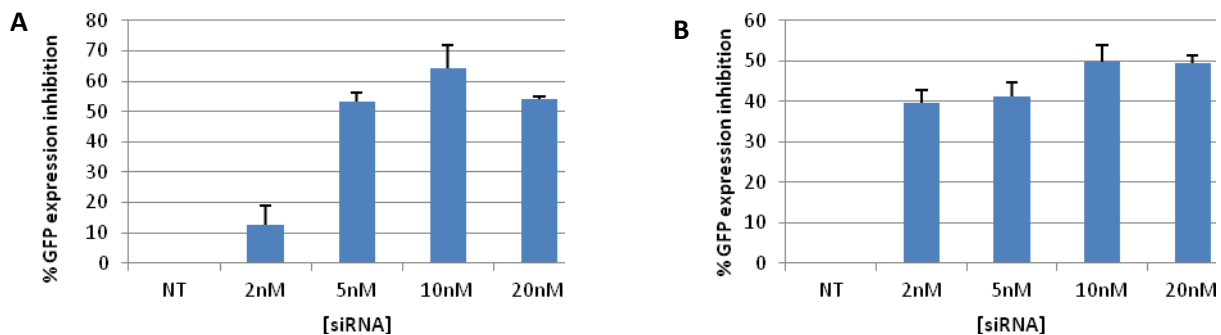


Figure 3: GFP-stably transduced human MSC (A) and AFSC (B) were assayed with **Lullaby® Stem** reagent and siRNA (targeting GFP). Cells were seeded in 24 well plate and transfected in 500µL of complete medium. GFP expression was monitored 72h after transfection. Results show the percentage of GFP expression inhibition.

Gene silencing time course

Gene silencing depends on your amount of siRNA used as well as incubation time. With low doses of siRNA, highest silencing rate have been obtained after 48h on MSC and AFSC (Fig. 4)

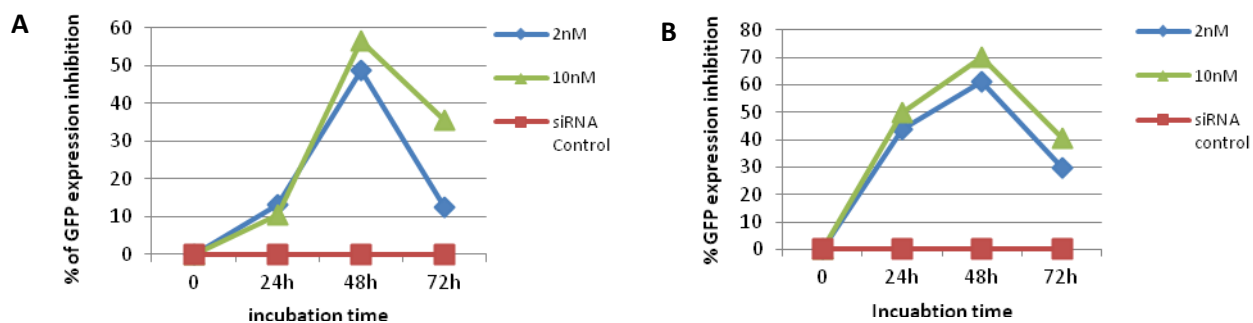


Figure 4: GFP-stably transduced human MSC (A) and AFSC (B) were assayed with **Lullaby® Stem** reagent and siRNA (targeting GFP). Cells were seeded in 24 well plate and transfected in 500µL of complete medium. GFP expression was monitored 24, 48 and 72h after transfection. Results show the percentage of GFP expression inhibition.

siRNA-fluorescein cellular uptake

Lullaby Stem exhibits high transfection efficiency on stem cells as shown by the delivery of a fluorescently-labelled siRNA (Fig. 5, Fig. 6).

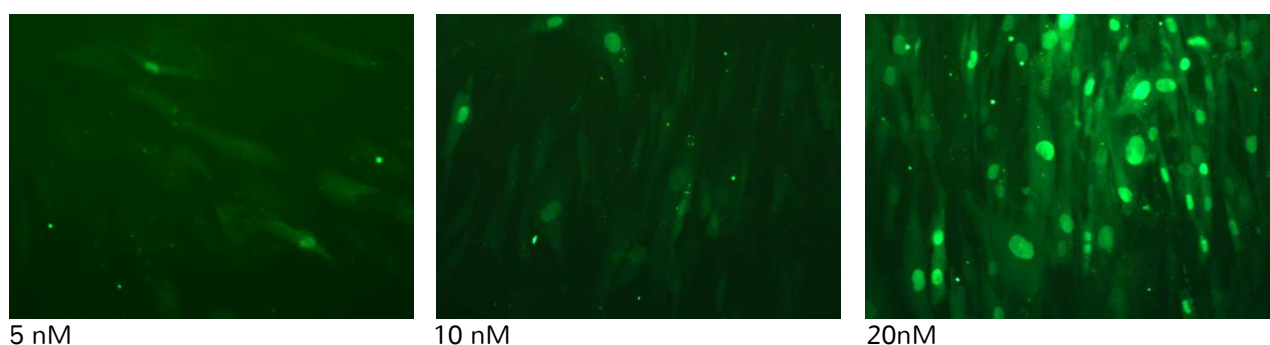


Figure 5: Representative pictures of human MSC 48h after treatment with **Lullaby® Stem** transfection reagent and increasing doses of fluorescently-labelled siRNA.

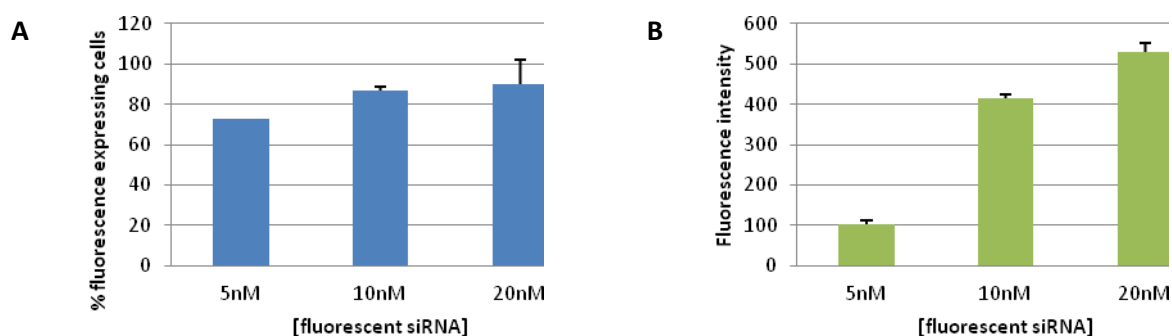


Figure 6: Human MSC were assayed with **Lullaby® Stem** transfection reagent and increasing concentrations of fluorescently-labelled siRNA. Cellular uptake (A, B) was monitored 24h after transfection by cytofluorimetry.

Comparison of Lullaby Stem with other siRNA transfection reagents

Lullaby Stem outperforms other transfection reagents in silencing gene expression in stem cells (Fig. 7). In addition, compared with other transfection reagents, no significant cytotoxicity was observed with **Lullaby® Stem** (Fig. 7).

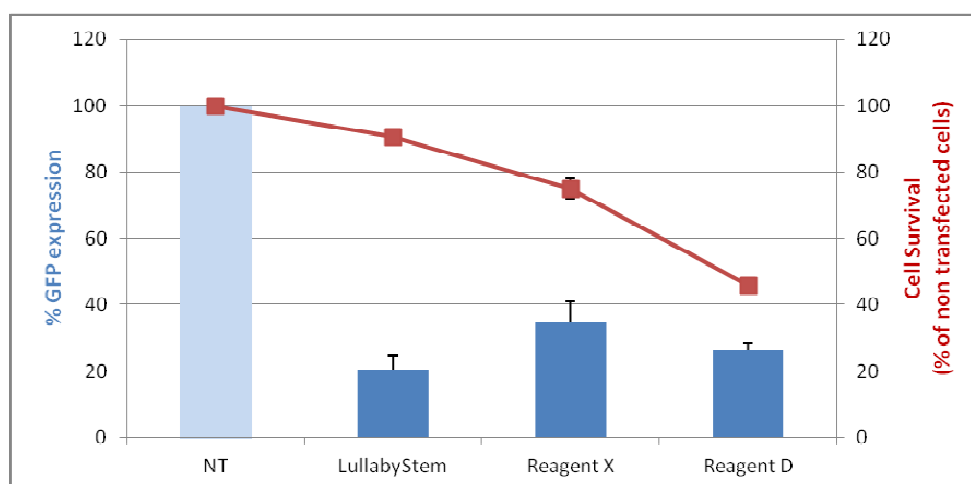


Figure 7: Human MSC stably expressing GFP were transfected with 10 nM of siRNA directed against GFP and Lullaby® Stem or with competitors' reagents according to manufacturers manuals. 72h after transfection, silencing efficiency was monitored by flow cytometry and % of living cells was analyzed by MTT assay.

Lullaby® Stem reagent induces high level of gene silencing in Stem cells through a very efficient transfer of siRNA into the cell cytoplasm in a non-toxic manner.

Stem Cells successfully tested with Lullaby® Stem: multipotent stem cells like Mesenchymal Stem Cells (MSC), Adipose-derived Stem Cells (AdSC), Amniotic Fluid Stem Cells (AFSC) human Embryonic Stem Cells (hESC) and 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.