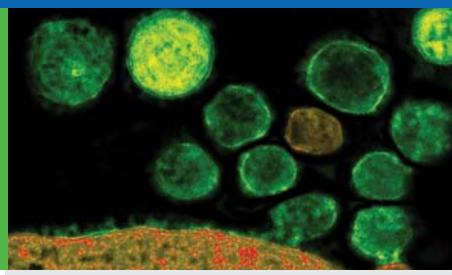
System Biosciences
Accelerating discoveries through innovations

Exosome Research

EV Shuttle Kits

Next generation transfection system using exosomes

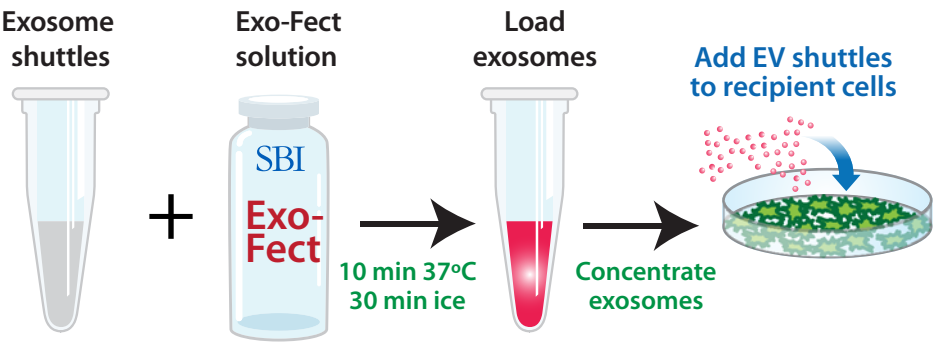


Exosomes are Nature's Effective Delivery Systems

Working with hard-to-transfect cells?
The EV Shuttle kit takes advantage of the natural ability of exosomes to be internalized and deliver functionally active biomolecules such as nucleic acids into recipient cells. Ready-to-use exosomes derived from human embryonic kidney cells (HEK 293) or murine dendritic cells (JAWS) can be transfected with nucleic acids including siRNA, miRNAs, mRNAs as well as plasmid DNA and added to a variety of hard-to-transfect cells for enhanced functional delivery. In addition, this technology can be used to create stable cell lines which is particularly useful for cells that are refractory to transfection using the piggyBac transposon system. EV shuttles also offer a significant advantage over virus-based transduction methodology because they are non-viral, easy to handle and cost effective method to deliver RNA and DNA into target cells.

The EV shuttle Kit contains a novel nucleic acid transfer agent (Exo-Fect) that enables the transfection of nucleic acids directly into isolated exosomes. The transfected si/miRNA, mRNA and even plasmid DNA can then be shuttled into target cells via the transfected exosome vesicles. Simply combine the isolated exosomes provided in the kit with Exo-Fect and the nucleic acid of your choice to generate exosome delivery vehicles. The protocol takes less than an hour and highly efficient at loading nucleic acids into exosomes for transport and delivery.

Easy Exosome Loading Protocol

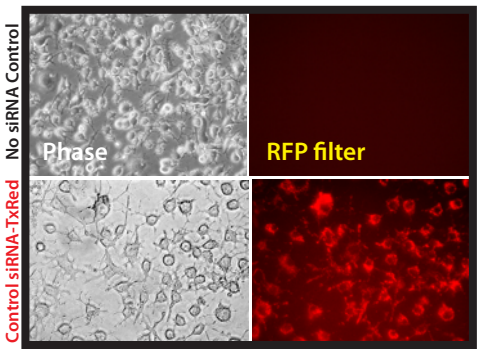


Mouse and Human EV shuttles deliver siRNA to hard-to-transfect cells. JAWS II mouse dendritic cell exosomes were Exo-Fected with 100 pmol Texas-Red conjugated siRNA (non-targeting control included in kits). The siRNA-loaded EV shuttles were then added to naive mouse monocyte macrophage cells (RAWS 264.7) in culture. The cells were imaged after 18 hours and delivery was observed as soon as 4 hours after adding the EV shuttles. More than 80% of the recipient RAWS 264.7 cells internalized the EV shuttle siRNA cargo (upper set of panels). Standard Lipofectamine® 2000 transfection protocols were also compared to Human EV shuttles on Raws 264.7 cells using the exact same amount of Texas-Red labeled siRNA (100 pmol, see lower panels). EV shuttles are more efficient at transfecting siRNA into transfection-resistant cells in culture.

Highlights

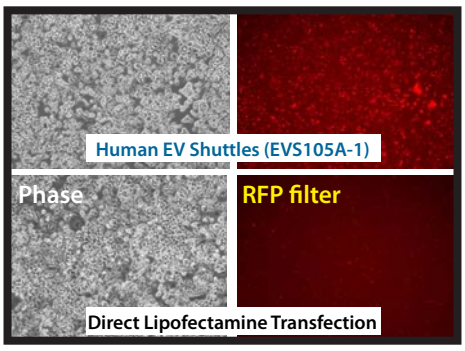
- Efficient delivery system of RNA and DNA delivery to transfection-resistant cells
- Simple loading protocol
- Non-viral delivery platform
- Create stable cell lines using EV shuttles
- Human and Mouse EV shuttles available

JAWS II EV shuttles added to RAWS 264.7 Cells (100 pmol siRNA)



Positive control non-targeting siRNA-Texas red label included in all EV shuttle kits

Lipofectamine versus Human EV shuttles siRNA transfection of RAWs cells
(100 pmol siRNA, 10x mag.)



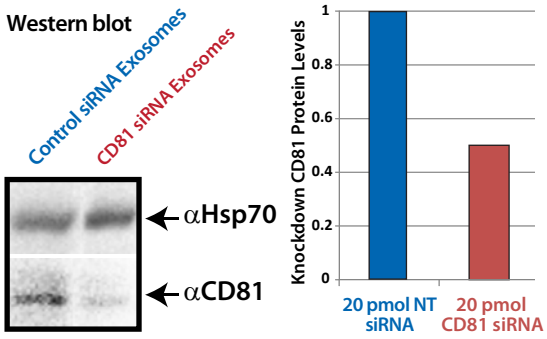
www.systembio.com/evshuttle

EV shuttle kits available

Human EV Shuttles	Catalog#	Size
EV Shuttle Kit Human HEK293 cell exosomes	EVS105A-1	5 Reactions
EV Shuttle Kit Human HEK293 cell exosomes	EVS110A-1	10 Reactions

Mouse EV Shuttles	Catalog#	Size
EV Shuttle Kit Mouse JAWS II bone marrow dendritic cell exosomes	EVS205A-1	5 Reactions
EV Shuttle Kit Mouse JAWS II bone marrow dendritic cell exosomes	EVS210A-1	10 Reactions

EV shuttles deliver functional siRNA

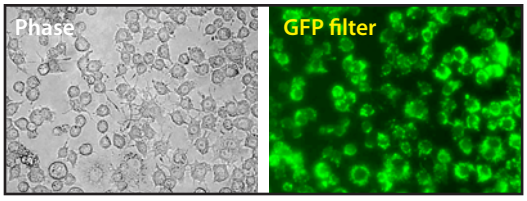


Knockdown genes in hard-to-transfect immune cells using EV shuttles!

Human EV shuttles can knockdown Mouse genes

Human HEK 293 EV shuttles were loaded with an anti-CD81 siRNA and then added to Mouse RAWS 264.7 cells. The cell lysates were collected after 18 hours for Western blot analysis of CD81 protein knockdown. Shown to the left, the Human EV shuttles carrying the anti-CD81 siRNA were able to knockdown the expression of the target cell's CD81 protein levels by at least 50% (Western image data were quantitated and graphed in the adjacent panel).

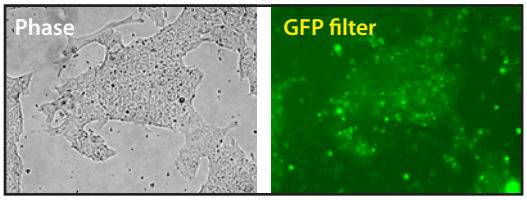
Mouse RAWS cells treated with Human EV shuttles



EV Shuttles deliver cross-species

Human HEK 293 EV shuttles were treated with SBI's Exo-Green kit (cat# EXOG200A-1) which labels exosome internal proteins fluorescently green. The labeled Human EV shuttles were then added to Mouse RAWS 264.7 macrophage cells and imaged for cargo delivery after 18 hours. The Human EV shuttles were well-tolerated and the EVs were taken up efficiently by the Mouse macrophage cells (above) as well as by Mouse embryonic stem cells (below).

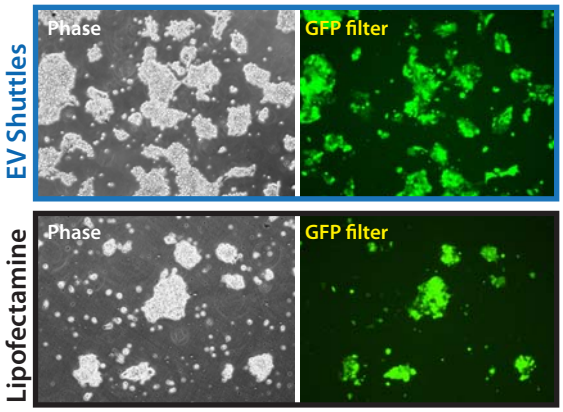
Mouse embryonic stem cells treated with Human EV shuttles



Mouse embryonic stem cells take up Human EVs

Make stable cell lines using EV shuttles and *piggyBac*

The *piggyBac* (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the Super PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The Super PB transposase expression plasmid (5 ug) and 5 ug of the PB713B-1 PB transposon vector encoding markers for GFP and a Puromycin resistance gene were used to compare the efficiency of transposition using either standard Lipofectamine methods or HEK293 EV shuttles. After 72 h post EV shuttle treatment, puromycin selection was initiated at 2 ug/ml and then increased to 5 ug/ml for 3 days further. EV shuttle-mediated transpositioned HEK293 cells were imaged post selection (upper panel) and compared to cells that were Lipofectamine transfected with the same PB plasmids (lower panel). Delivery of *piggyBac* vectors by EV shuttles was less toxic than transfection, highly efficient at PB transposon host chromosome integration, and resulted in approximately 5-times more colonies.



EV Shuttles have less cytotoxicity while generating more stable cell line colonies.

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