

that efficiently delivers both DNA *and* RNA out of the endosome and into the cytoplasm, overcoming a critical barrier to nucleic acid delivery.

The *Trans*IT-X2® Dynamic Delivery System gives researchers:

- **Efficiency**—exceptional broad spectrum transfection
- Delivery—simultaneous delivery of plasmid DNA and siRNA
- Technology—novel, non-liposomal, polymeric technology

"We recently tested *Trans*IT-X2® Dynamic Delivery System head-to-head against Lipofectamine® 2000 for DNA transfection of NIH-3T3 fibroblasts and the breast cancer cell line ZR-75-1. We observed higher efficiency and less toxicity when using *Trans*IT-X2®. We are also pleased to hear that *Trans*IT-X2® will be offered in similar volume configurations to Lipofectamine® 2000."

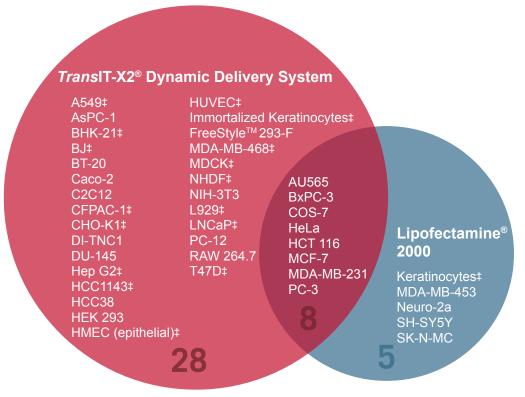
-Dr. Edwin Li, Assistant Professor, St. Joseph's University





TransIT-X2® Dynamic Delivery System

Outperforms Lipofectamine® 2000 in 28 Cell Lines



‡ Cell types with >2-fold luciferase expression in head-to-head comparisons.

Figure 1. TransIT-X2® Dynamic Delivery System enables superior gene expression in a variety of cell types. TransIT-X2® Dynamic Delivery System and Lipofectamine® 2000 Transfection Reagent were used to transfect plasmid DNA encoding luciferase into 41 different cell types at three reagent-to-DNA ratios. Luciferase expression was compared at 24 hours post-transfection using a standard luciferase assay. Head-to-head comparisons at optimized ratios illustrate superior or equal luciferase expression using TransIT-X2® in 36 of 41 cell types; 16 cell types had expression levels 2-fold higher than Lipofectamine® 2000 (denoted with ‡).

Co-delivery of Plasmid DNA and siRNA

2

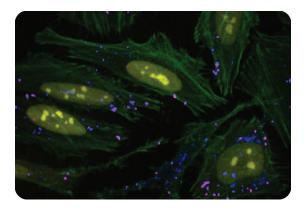
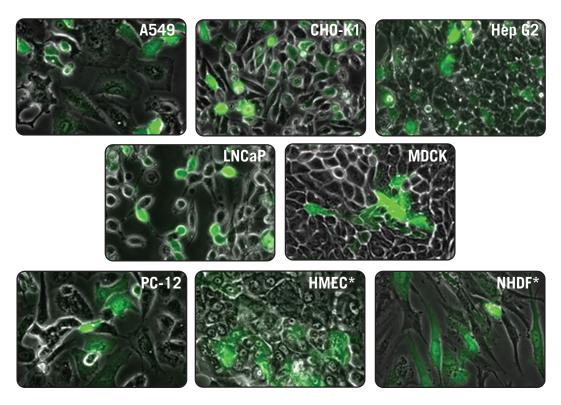


Figure 2. Functional co-delivery of plasmid DNA and siRNA using TransIT-X2® Dynamic Delivery System. TransIT-X2® Dynamic Delivery System was used to transfect Cy^{TM5} labeled DNA encoding nuclear YFP and Cy^{TM3} labeled siRNA into HeLa cells. Transfection was performed in a 6-well plate with Poly-L-Lysine (PLL) coated coverslips using 4 μ l of TransIT-X2® to deliver 2 μ g of DNA (2:1 reagent:DNA ratio) and 25nM siRNA. Actin cytoskeleton was stained using Alexa Fluor® 350 Phalloidin. Image (63X) was captured at 24 hours post-transfection using a Nikon A1R confocal microscope. Merged image key: yellow (nuclear YFP), blue (Cy5 labeled DNA), red (Cy3 labeled siRNA), green (actin cytoskeleton).

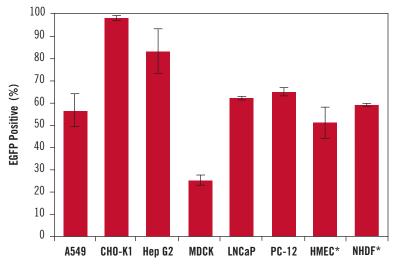


Outstanding GFP Efficiency Using TransIT-X2® Dynamic Delivery System



*indicates primary cell types

Figure 3. Visualization of high GFP expression using TransIT-X2® Dynamic Delivery System. TransIT-X2® Dynamic Delivery System was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, LNCaP, MDCK, PC-12, primary human mammary epithelial cells (HMEC) and normal human dermal fibroblasts (NHDF). Transfections were performed in 35 mm MatTek dishes using 4-8 μl of TransIT-X2® to deliver 2 μg of DNA. Images (32X) were captured at 48 hours post-transfection using a Zeiss Axiovert S100 inverted fluorescence microscope.



*indicates primary cell types

Figure 4. High GFP transfection efficiency in multiple cell lines and primary cells using *Trans*IT-X2® Dynamic Delivery System. *Trans*IT-X2® Dynamic Delivery System was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, MDCK, LNCaP, PC-12, primary human mammary epithelial cells (HMEC) and normal human dermal fibroblasts (NHDF). Transfections were performed in 96-well plates using 0.2-0.4 μl of *Trans*IT-X2® to deliver 0.1 μg of DNA (2:1, 3:1 or 4:1 reagent: DNA ratio). Triplicate wells were assayed 48 hours post-transfection using a guava easyCyte™ 5HT Flow Cytometer.

TransIT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000

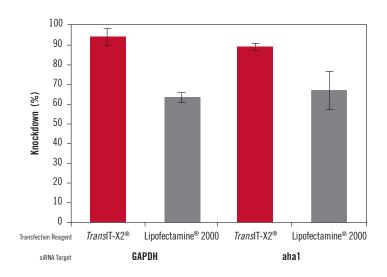


Figure 5. *Trans*IT-X2® **Dynamic Delivery System achieves higher knockdown than Lipofectamine® 2000.** *Trans*IT-X2® Dynamic Delivery System and Lipofectamine® 2000 Transfection Reagent were used to transfect siRNA targeting endogenous proteins - GAPDH, aha1 or non-targeting control in primary normal human dermal fibroblasts (NHDF). Cells were transfected in a 6-well plate using 4 μl of *Trans*IT-X2® or 6 μl of Lipofectamine® 2000 and 25nM siRNA according to each manufacturer's protocol. The amount of GAPDH or aha1 mRNA was measured relative to 18s rRNA levels using qRT-PCR and then normalized to the mRNA levels of the no-targeting control, 48 hours post-transfection. Error bars represent the standard deviation of triplicate wells.

Effective miRNA Delivery Using TransIT-X2® Dynamic Delivery System

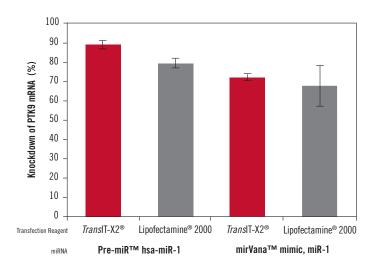


Figure 6. Effective miRNA delivery using TransIT-X2® Dynamic Delivery System yields decreased levels of PTK9 mRNA. TransIT-X2® Dynamic Delivery System and Lipofectamine® 2000 Transfection Reagent were used to transfect T47D cells with Pre-miR™ hsa-miR-1 miRNA Precursor or mirVana™ miRNA mimic, miR-1, both known to decrease PTK9 mRNA levels. A Pre-miR negative control was transfected to assess baseline mRNA levels. Cells were transfected in a 12-well plate using 3 µl of TransIT-X2® or Lipofectamine® 2000 and 50 nM miRNA according to each manufacturer's protocol. The amount of PTK9 mRNA was measured relative to 18s rRNA levels using qRT-PCR and then normalized to the mRNA levels of the negative control, 48 hours post-transfection. Error bars represent the standard deviation of triplicate wells.

Broad Spectrum DNA and siRNA/miRNA Transfection

Product Name	Product No.	Quantity
TransIT-X2®	MIR 6003	0.3 ml
Dynamic Delivery System	MIR 6004	0.75 ml
	MIR 6000	1.5 ml
	MIR 6005	5 X 1.5 ml
	MIR 6006	10 X 1.5 ml

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