*Trans*IT-TKO® Transfection Reagent

A high efficiency, low toxicity, siRNA transfection reagent for mammalian cells

- **Broad Spectrum siRNA Delivery**—Utilize one transfection reagent and protocol for a wide variety of cells.
- **Low Cellular Toxicity**—Maintain cell density and reduce experimental biases.
- **Reproducible Results**—Obtain consistent, targeted gene knockdowns from day to day.
- **High Knockdown Efficiency**—Achieve optimal gene silencing in a large percentage of cells to ensure experimental success.

Cell Types Successfully Transfected by Mirus Bio:
A549, BHK-21, BNL.CL2, C2C12, C6, CHO-KI, COS-7, Daoy, DB-TRG-05MG, D1-TNC1, DU145, HEK 293, HeLa, Hepa1c1c7, HepG2, human astrocytes, Jurkat, Keratinocytes (NIKS), MCF-7, Neuro-2a, NIH 3T3, PC-3, primary mouse hepatocytes, RAW 264.7, SK-N-MC, THP-1, Vero.

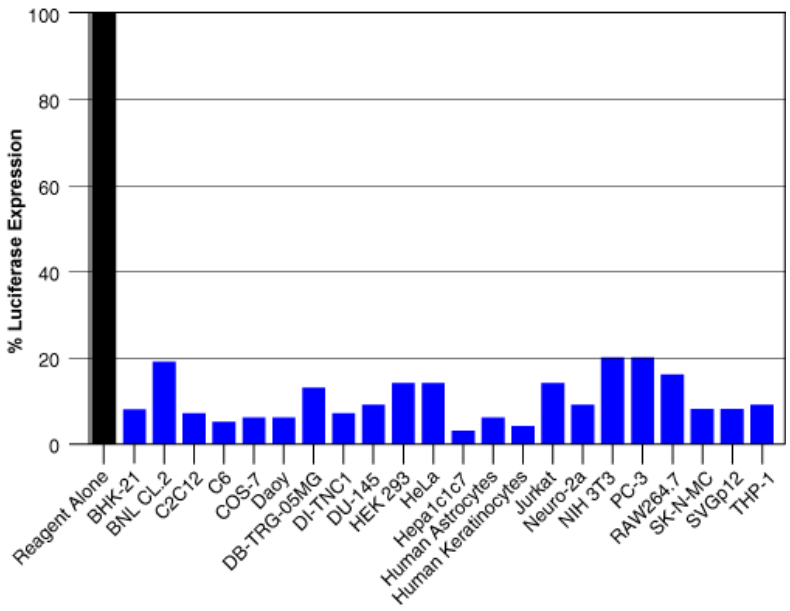

Figure 1.

Efficient Target Gene Knockdown in Selected Cell Lines Using *Trans*IT-TKO® Reagent. Reporter plasmids expressing both firefly and sea pansy luciferases were co-transfected into the indicated cell lines using *Trans*IT® Plasmid Transfection Reagents. Targeted knockdown was achieved by transfection of an anti-firefly luciferase siRNA using the

*Trans*IT-TKO® Reagent. Twenty-four hours later, firefly luciferase expression was normalized to sea pansy luciferase expression and compared to the reagent alone control.

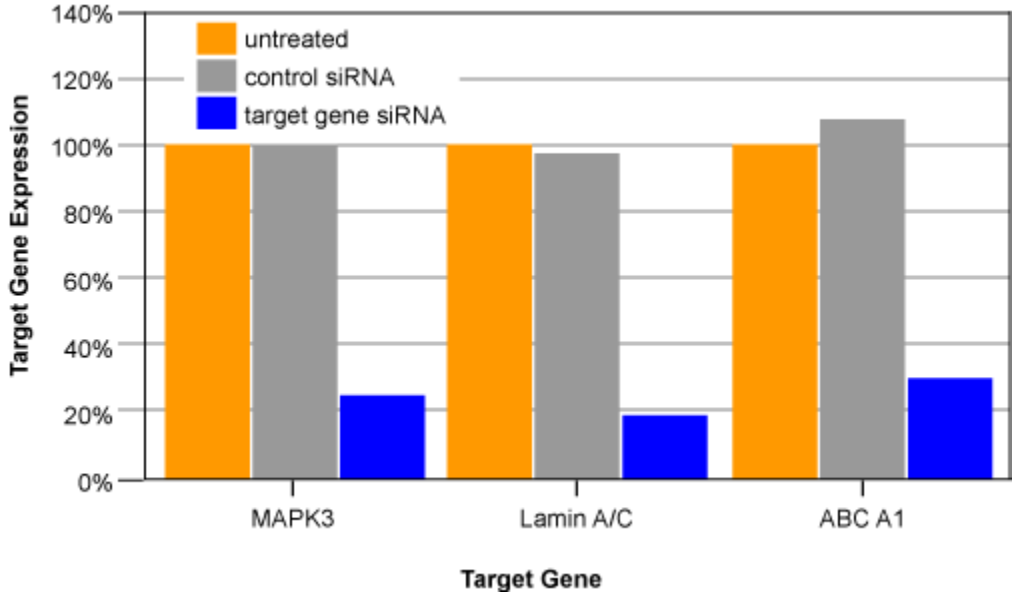

Figure 2. Efficient Knockdown of Endogenous Genes in Primary Hepatocytes Using *Trans*IT-TKO® Reagent. Primary mouse hepatocytes were transfected with the indicated siRNAs or a non-targeting control siRNA using the *Trans*IT-TKO® Reagent. Twenty-four hours post-transfection, the amount of each mRNA was measured relative to GAPDH mRNA levels using qRT-PCR and then scaled to the expression level of the specific target mRNA in the cells alone (untreated) controls.

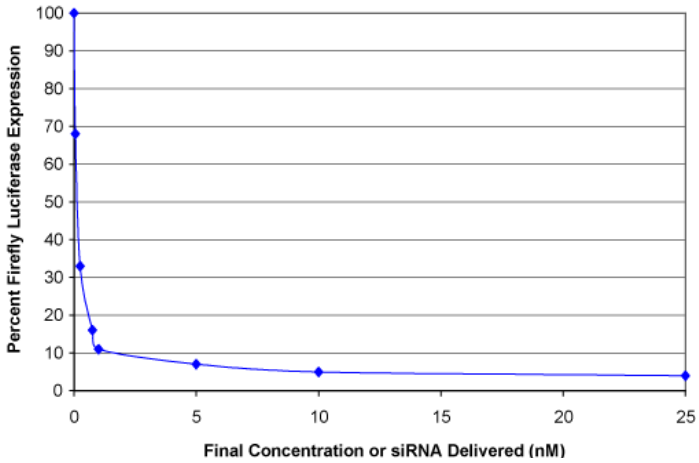

Figure 3. Efficient Knockdown of Luciferase Expression Using *Trans*IT-TKO® Reagent to Deliver Nanomolar Amounts of siRNA. Reporter plasmids expressing firefly and sea pansy luciferase were transfected into COS-7 cells using the *Trans*IT®-LT1 Reagent. After four hours, various concentrations of anti-firefly luciferase siRNA (0, 0.05, 0.25, 0.75, 1, 5, 10, and 25 nM) were complexed with the *Trans*IT-TKO® Reagent and transfected into COS-7 cells in

their complete media. Twenty-four hours post-transfection, firefly luciferase expression was normalized to sea pansy luciferase expression and compared to the reagent alone control.

Cell Line (Source)	Endogenous Transcipt	Knockdown Efficiency
BNL CL.2 (mouse liver)	MAPK1	80%
	MAPK3	83%
HeLa (human cervix)	Lamin A/C	80%
	GAPDH	80%
Hepa1c1c7 (mouse liver)	MAPK1	80%
	MAPK3	75%
	MEK1	75%
	PTEN	80%
HepG2 (human liver)	MAPK1	80%
NIH 3T3-L1	MAPK1	70%
	MAPK3	70%
Secondary Human Astrocytes	Lamin A/C	80%
Primary Mouse Hepatocytes	ABC A1	70%
	Lamin A/C	81%

Figure 4. Knockdown of Endogenous Genes Using *Trans*IT-TKO® Reagent. Various cells were transfected with siRNAs targeting the indicated genes using the *Trans*IT-TKO® Reagent, and the knockdown percentage was determined using quantitative RT-PCR.

Figure 5. Visualization of Fluorescently Labeled siRNA. siRNA was fluorescently labeled with the *Label* IT® siRNA Tracker Fluorescein Kit and transfected into HeLa cells using the *Trans*IT-TKO® Reagent. At 24 hours, cells were fixed, counterstained, and analyzed by confocal microscopy to visualize the siRNA (green), nuclei (blue), and actin (red).