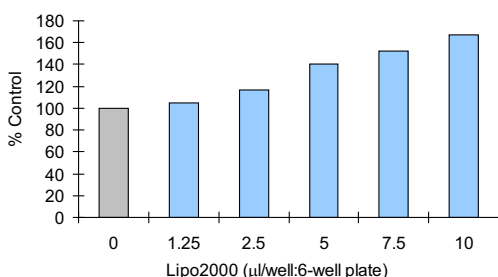


Are you really transfecting DNA with the original cell condition?

The most popular transfection reagent Lipofectamine2000 contains effective components which enhance protein expression.

Recently, some researchers suggested that the incomprehensible results were obtained using Lipofectamine2000 (Lipo2000) in immune response research.

Based on a published paper¹⁾, eight ninths of the affected genes related to immune responses were up-regulated using Lipo2000. The up-regulated genes were especially related with IFN or cytokine. This result suggests that Lipo2000 may induce a non-specific cellular immune response.



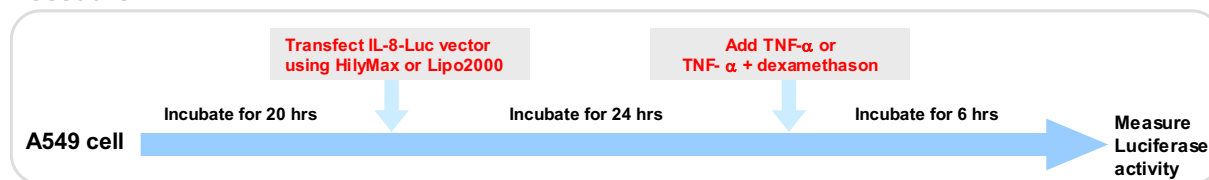
The changed protein expression was monitored by adding Lipo2000 using GFP expressing cells (HT1080 cell).

The GFP expressing cells were used to analyze the amount of expressed GFP after the addition of Lipo2000 by flowcytometry. The results show that GFP expression was dose-dependent increase in Lipo2000.

Good Signal Transduction Responses with HilyMax Transfection

The signal transduction in the A549 cell was confirmed with TNF- α stimulation. For detection of cellular response, the IL-8 dependent luciferase expression vector was transfected with HilyMax or Lipo2000. The signal transduction response was detected as luciferase activity after stimulation and suppression.

Procedure



Result

Over expression of IL-8 was observed without TNF- α stimulation using Lipo2000.

When the cell is not stimulated, the luciferase activity should be low. However the luciferase activity of the cell transfected by Lipo2000 indicates high luciferase activity. On the other hand, the cell transfected by Dojindo's HilyMax produced a much lower luciferase activity.

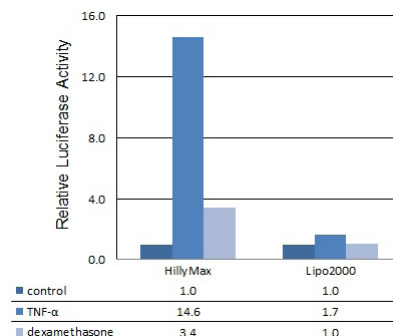
Higher response of signal transduction was observed when HilyMax was used.

Increased Luciferase Activity by TNF- α HilyMax : x14.5 Lipo2000: x1.7

Suppressed Luciferase Activity by Dexamethason HilyMax : 77% Lipo2000: 59%

When the signal response of IL-8 was monitored without transfection, the amount of IL-8 was 10 times increased after addition of TNF- α in A549 cells²⁾. More reliable data from the transduction assay was obtained by HilyMax transfection.

(Data was kindly provided from Dr. Yoichiro Isohama, Kumamoto University, Faculty of Medical and Pharmaceutical Sciences, Dept. of Chemo-Pharmacological Sciences)



¹⁾ Tatsuaki Tagami, 1 Kiyomi Hirose, 1 Jose Mario Barichello, 1,2 Tatsuhiro Ishida, 1,3 and Hiroshi Kiwada1, "Global Gene Expression Profiling in Cultured Cells Is Strongly Influenced by Treatment with siRNA-Cationic Liposome Complexes", *Pharmaceutical Research*, **2008**, 25, 2497.

²⁾ Hironori Takei, Yuichiro Baba, Akinori Hisatsune, Hiroshi Katsuki, Takeshi Miyata, Kazumi Yokomizo, and Yoichiro Isohama, "Glycyrrhizin Inhibits Interleukin-8 Production and Nuclear Factor- κ B Activity in Lung Epithelial Cells, but Not Through Glucocorticoid Receptors", *J Pharmacol Sci.*, **2008**, 106, 460.