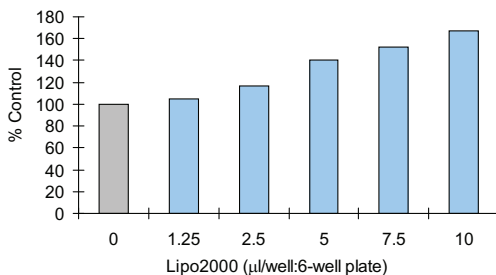


Are you really transfecting DNA with the original cell condition?

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

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

Based on a published paper¹⁾, eight-ninths of affected genes relating 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 the addition of 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 result show that GFP expression was dose-dependent increase in Lipo2000.

Good Signal Transduction Response with HilyMax Transfection

The signal transduction in A549 cell was confirmed with the TNF- α stimulation. For detection of cellular response, 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, luciferase activity should be low. However the luciferase activity of the cell transfected by Lipo2000 indicated a high luciferase activity. On the other hand, the cell transfected by Dojindo's HilyMax produce the much lower luciferase activity.

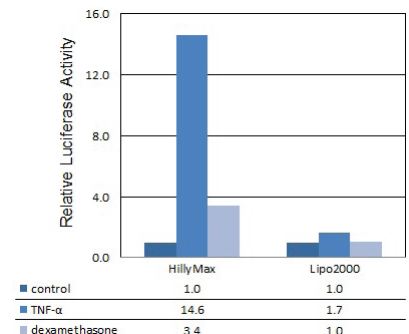
Higher response of signal transduction was observed when HilyMax was used.

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. Youichiro 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.