life technologies™

Lipofectamine® transfection reagents

Lipofectamine® transfection reagents shipped at ambient temperature reduce environmental impact and retain their quality and stability

Abstract

In order to minimize the adverse environmental impact of packaging and shipping products on gel ice, Life Technologies investigated the feasibility of shipping its Lipofectamine® transfection reagents at ambient temperature. This report describes stability and performance testing of two classes of Lipofectamine® transfection reagents (Lipofectamine® RNAiMAX and Lipofectamine® 2000) subjected to ambient shipping conditions. Our results indicate that Lipofectamine® 2000 and RNAiMAX reagents can withstand the rigors of ambient shipping in both summer and winter conditions, without impacting the stability or functionality of the products. By shipping at ambient conditions, we eliminate the environmental impact of shipping product in expanded polystyrene (EPS, also known as Styrofoam®) coolers laden with frozen gel packs.

Introduction

The adverse environmental impact of shipping refrigerated products is tremendous. The annual carbon footprint to manufacture EPS and convert it into coolers for Life Technologies Lipofectamine® transfection reagents is approximately 40 tons (CO_2-equivalents). It takes nearly 120 barrels of crude oil equivalents and 150 MW-hr of power annually to make these EPS coolers. Additionally, 30,000 liters of water are consumed in the manufacture of the EPS coolers.

An average of 8 pounds of frozen gel packs is added to each cooler to ensure the product is delivered refrigerated to our customers, further increasing the mass and dimensions of each package. Factoring in the number of shipments and average distance traveled per package, an additional 40 tons (CO_2-equivalents) of greenhouse gases are generated from the transport of the coolers every year. By combining the elimination of EPS and reduction in impact attributed to transporting the added weight of the frozen gel packs, we reduce the annual total carbon footprint from product delivery by over 80 tons (CO_2-equivalents). This is equivalent to the emissions from 9 homes every year.

Life Technologies has been systematically evaluating novel ways to minimize the environmental impact of shipping refrigerated/frozen products. One way to achieve this is to ship our products at temperatures consistent with their demonstrated stability. By eliminating the need for a cooler and refrigerant, products can be shipped in smaller boxes, improving the carrier's freight density (less fuel and emissions per box) while reducing consumption of raw materials to make the packaging. This enables Life Technologies to eliminate an annual total of nearly 9000 kg (24,000 ft^3) of EPS from our customer's landfills and incinerators and replace it with recyclable corrugated paper packaging.

For many years, Life Technologies has shipped our Lipofectamine® transfection reagents in coolers filled with frozen gel packs. The frozen gel packs prevent products from being exposed to extreme temperatures associated with shipping. New data, described herein, have demonstrated that these reagents are not impacted when subjected to simulated ambient shipping conditions.

This paper describes the results from the functional and analytical tests conducted on Lipofectamine® 2000 and Lipofectamine® RNAiMAX reagents that were exposed to established summer and winter shipment simulation profiles. These experiments demonstrate that by shipping our Lipofectamine® transfection reagents under ambient conditions, not only can we supply researchers with the same superior-quality product they are used to receiving, but we can reduce the environmental footprint of delivering this product. This is a win for the planet (reducing wasteful resource consumption and total carbon footprint), and a win for our customers (minimizing packaging waste).

Materials and methods

Test product and size

Test articles were obtained from multiple dispensed lots taken from inventory. Standard primary (tubes) and secondary (folded paper) kit packaging were used. All materials were tested in validated environmental chambers without tertiary (corrugate box) packaging to minimize insulation. Test articles are described in Table 1.

Table 1. Lipofectamine® transfection test articles and controls

Product	Cat. No.	Size
Lipofectamine® RNAiMAX	13778-075	0.75 mL
Lipofectamine® RNAiMAX	13778-100	0.1 mL
Lipofectamine® RNAiMAX	13778-150	1.5 mL
Lipofectamine® 2000 Transfection Reagent	11668-027	0.25 mL*
Lipofectamine® 2000 Transfection Reagent	11668-019	1.5 mL

***Because product volume has an impact on thermal mass, we removed 0.5 mL aliquots of Lipofectamine® 2000 (Cat. No. 11668-027) to create a "worst case" scenario for shipping simulations and accelerated stability testing.**

Simulated shipping

To simulate temperatures potentially incurred during shipping, we based our study on summer and winter models previously developed at Amgen Corporation [3]. These models were validated against 2,500 shipments between the latitudes of 59.9° north and 37.8° south.

We selected the Amgen models over the ISTA 7E Standard [4] because they were more rigorous in temperature extremes and maintained a comparable Mean Kinetic Temperature. Both the summer and winter simulations were duplicated (back to back) to create a "worst case" scenario (Figures 1 and 2).

Accelerated stability design

Based on historical knowledge of the Lipofectamine® 2000 and Lipofectamine® RNAiMAX reagents, we applied the Q Rule to predict product stability post ambient shipping simulation [1,2]. The Q Rule states that a product degradation rate decreases by a constant factor (Q_{10}) when the storage temperature is lowered by 10°C. The value of

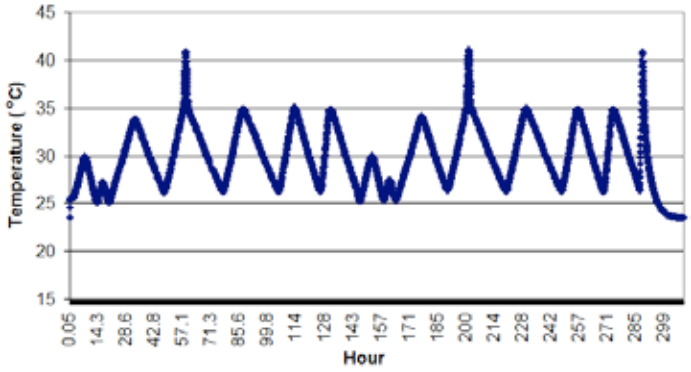

Figure 1. Actual profiles for summer simulation

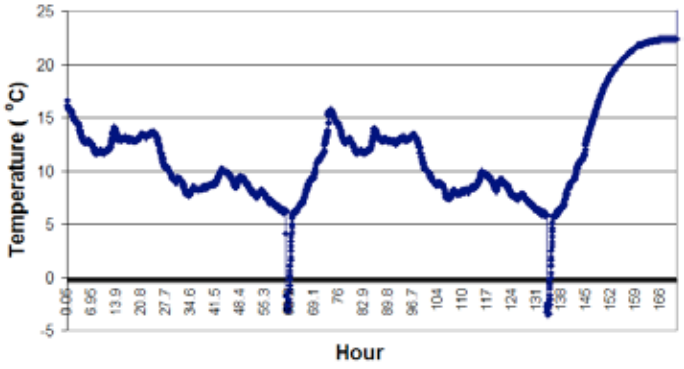

Figure 2. Actual profiles for winter simulation

Q_{10} was set at 2, corresponding to a conservative prediction of the activation energy required for product degradation.

Predicted Stability† = Accelerated Stability x $(Q_{10})^{\Delta T/10}$

The accelerated stability testing was performed with both control and test articles at +24°C at time points as indicated in Tables 2 and 3. Controls were not subjected to ambient shipping simulation, but kept at +4°C for testing at t = 0 and at +24°C together with test articles. At each time point, test and control products were evaluated side by side.

Table 2. Accelerated stability testing schedule

Post ambient shipping simulation (weeks)	Performance evaluation		
	Particle size	LC-MS	Transfection efficiency
0	+	+	-
1	-	-	-
2	-	-	-
3	-	-	-
4	+	+	-
5	-	-	-
6	-	-	-
7	-	-	-
8	+	+	+

+ Indicates testing performed
- Indicates testing not performed

Table 3. Sampling plan

Methods	Ambient simulation	Sample type	Sample number for accelerated stability		
			Week 0	Week 4	Week 8
HPLC-MS	Summer	Test	2	0	2
	Winter	Test	2	0	2
		Control	2	0	2
Particle size	Summer	Test	4	4	4
	Winter	Test	4	4	4
		Control	4	4	4
Transfection efficiency	Summer	Test	0	0	4
	Winter	Test	0	0	4
		Control	0	0	4
Total			12	12	36

Testing methodology

Both test and control products were evaluated functionally and analytically.

Liquid chromatography-mass spectroscopic analysis
HPLC coupled with MS was used to evaluate the structural stability of the formulated products.

Particle size and distribution analysis
Particle analysis included the characterization of liposome size and distribution.

Table 4. Cell-line and transfection reagent

Cell line	Cell type	Species	Reagents
CHO-K1	Ovary	Hamster	Lipofectamine® 2000
COS-7	Kidney	Monkey	Lipofectamine® 2000
HEK293	Embryonic kidney	Human	Lipofectamine® 2000
HeLa	Cervical cancer	Human	Lipofectamine® 2000
MCF-7	Breast cancer	Human	Lipofectamine® 2000
HepG2	Hepatocellular carcinoma	Human	Lipofectamine® 2000
293-luc	Embryonic kidney	Human	Lipofectamine® RNAiMAX

Functional transfection efficiency analysis
Transfection efficiency with Lipofectamine® 2000 reagent was measured via expression of Green Fluorescent Protein (GFP) against a titration of reagent with 100 ng pcDNAEF1a/emGFP DNA per well in multiple cell lines. Expression (% of GFP-positive cells) was measured using fluorescence-activated cell sorting (FACS). Transfection efficiency with Lipofectamine® RNAiMAX reagent was measured via expression of siRNA against a titration of reagent with 10 nM Stealth RNAi® duplex, targeting luciferase and a negative control Stealth RNAi® duplex in 293-luc, a stably transformed cell line expressing the luciferase reporter gene (*luc*). Luciferase expression was measured using a Promega luciferase assay reagent (Part Number E1483) and a Mithras LB 940 luminometer.

Acceptance criteria

For all the products tested, the results must meet the specification or criteria as summarized in Table 5.

Table 5. Acceptance criteria

Method	Acceptance criteria
HPLC-MS	All test articles must be substantially free of degradation products compared to controls.
Particle size distribution	Particle size distribution for all test articles must be statistically equivalent to controls.
Transfection efficiency	Transfection efficiencies of test articles are statistically equivalent to controls.

Statistical analysis

Particle size and distribution was statistically compared using output from the JMP statistical program (JMP 8.0,

SAS Institute, Cary, NC 27513) running under Microsoft Windows XP, Service Pack 3). Triplicate analysis of duplicate samples from each lot was used to evaluate for equivalency between control and ambient simulation data.

Results and discussion

Liquid chromatography and mass spectroscopy

Product integrity changes in test samples compared to controls were assessed by HPLC and MS. There were no significant differences in the chromatograms between the 0- and 8-week samples as well as between the non-stressed controls and ambient shipped samples for both Lipofectamine® 2000 and RNAiMAX products (data not shown).

Particle size and distribution

Particle size was determined to evaluate if there were significant changes or differences in liposome size from the test articles and controls. There were no significant differences in the liposome size and their distribution between the non-stressed controls and ambient-shipped samples for both Lipofectamine® 2000 and Lipofectamine® RNAiMAX products (one-way analysis, Student's t-test, 95% confidence interval, data not shown).

Transfection efficiency

Transfection efficiency of Lipofectamine® 2000, measured as expression of GFP, was determined against a titration of the reagent in multiple cell lines as described above. The amount of plasmid was held constant while the amount of transfection reagent was titrated.

Titration series for multiple cell lines are shown in Figure 3–14, and statistical analysis of the normalized expression is illustrated in Figures 4, 6, 8, 10, 12, and 14. There were no significant differences among summer, winter, and control samples.

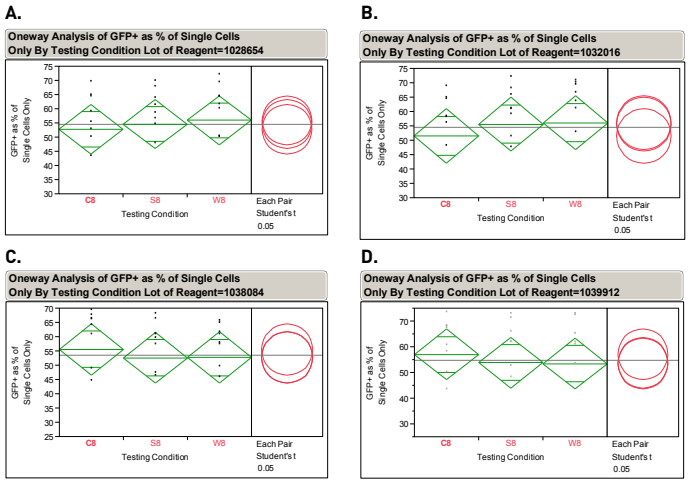

Figure 4. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent (C8, control; S8, summer; W8, winter) in CHO-K1. (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084 , (**D**) Lot Number 1039912.

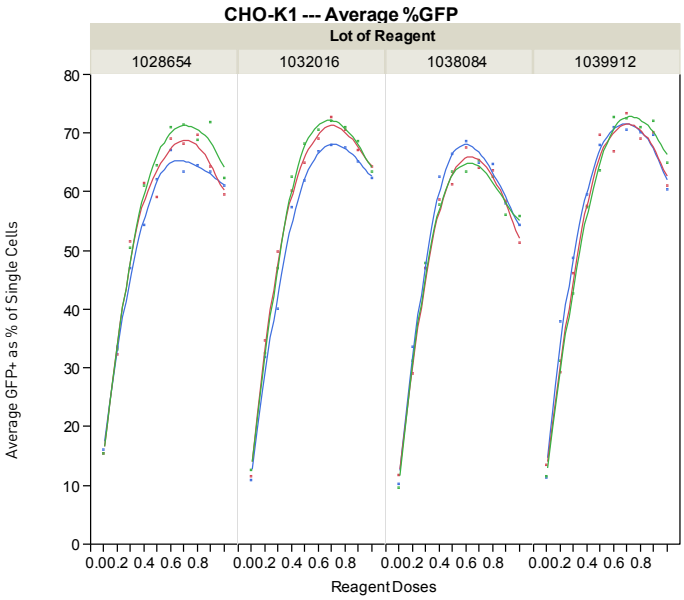

Figure 3. Titration series in CHO-K1 (average %GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

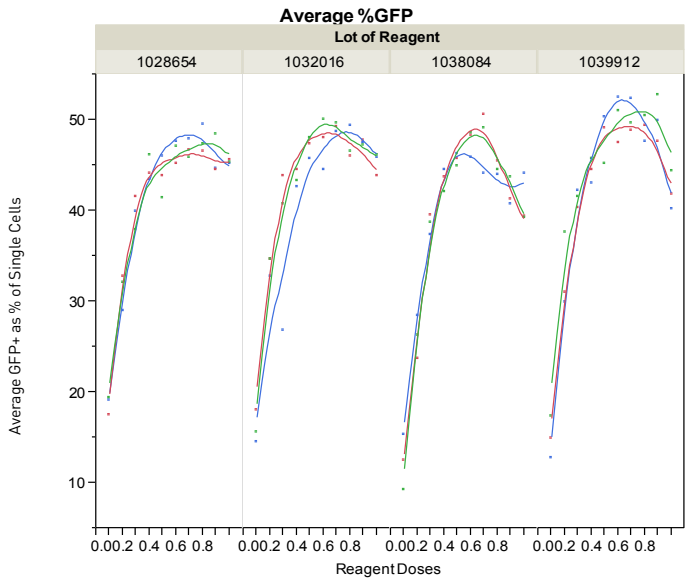

Figure 5. Titration series in COS-7 (average %GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

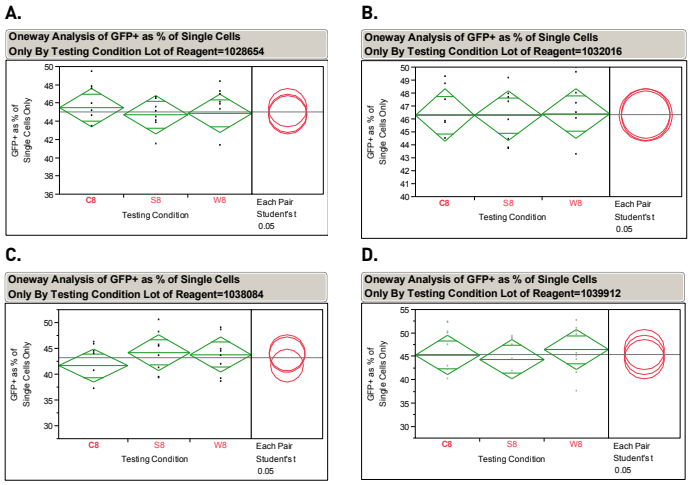

Figure 6. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent in COS-7 (C8, control; S8, summer; W8, winter). (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084, (**D**) Lot Number 1039912.

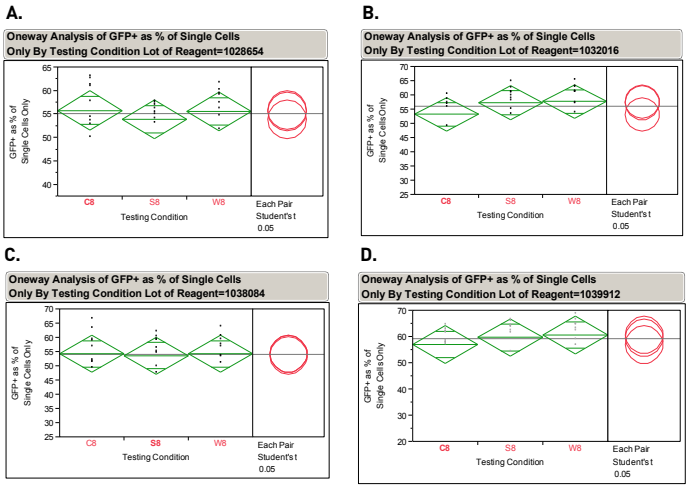

Figure 8. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent in HEK-293 (C8, control; S8, summer; W8, winter). (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084, (**D**) Lot Number 1039912.

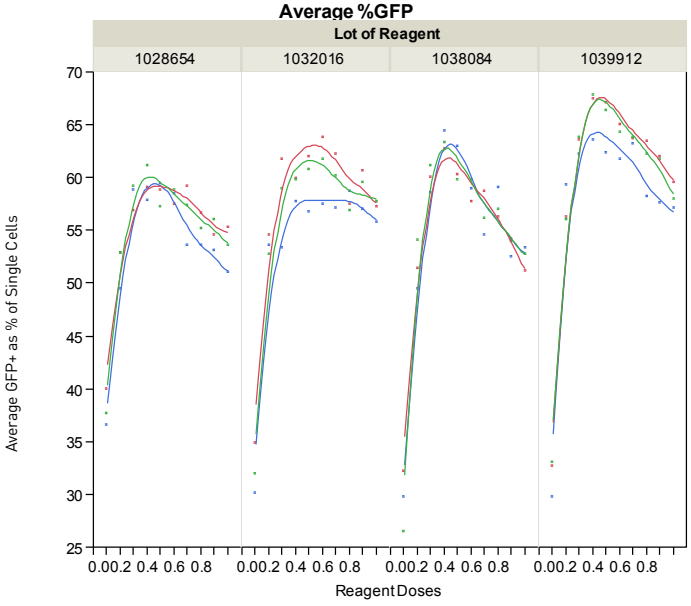

Figure 7. Titration series in HEK-293 (average %GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

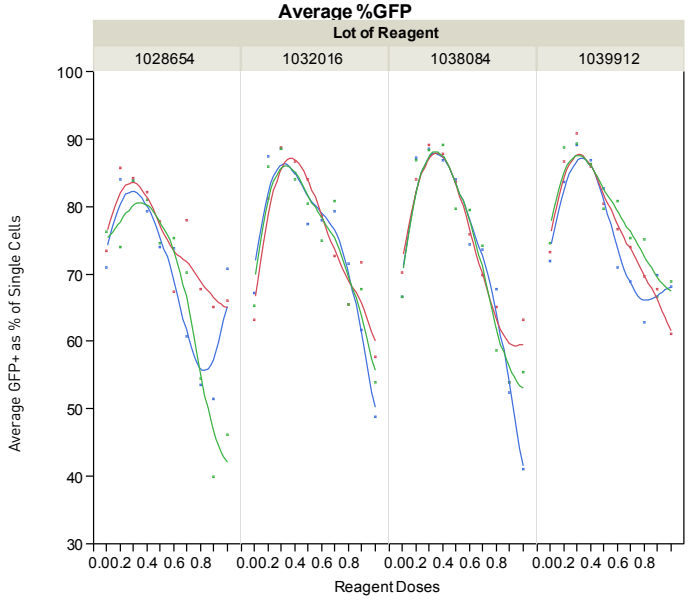

Figure 9. Titration series in HeLa (average %GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

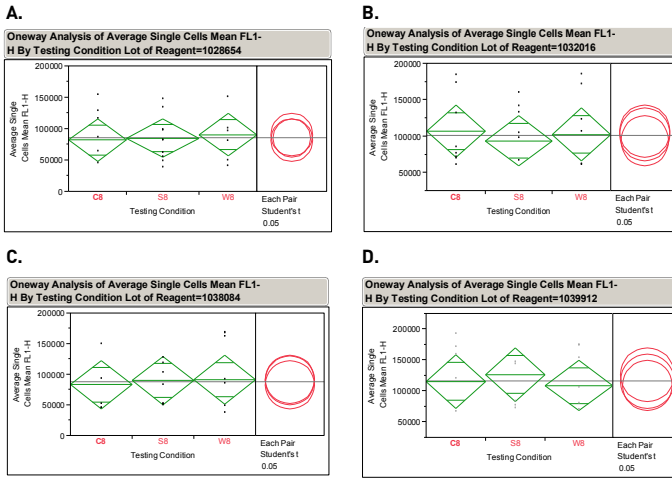

Figure 10. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent in HeLa (C8, control; S8, summer; W8, winter). (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084, (**D**) Lot Number 1039912.

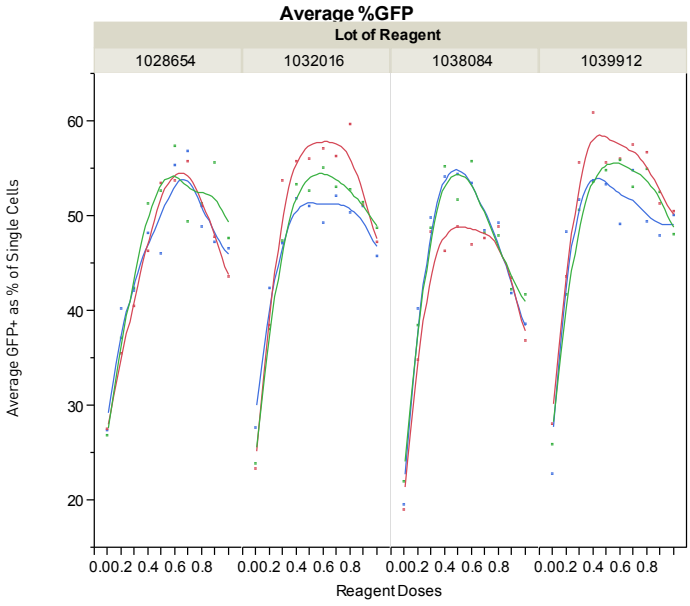

Figure 11. Titration series in MCF-7 (average % GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

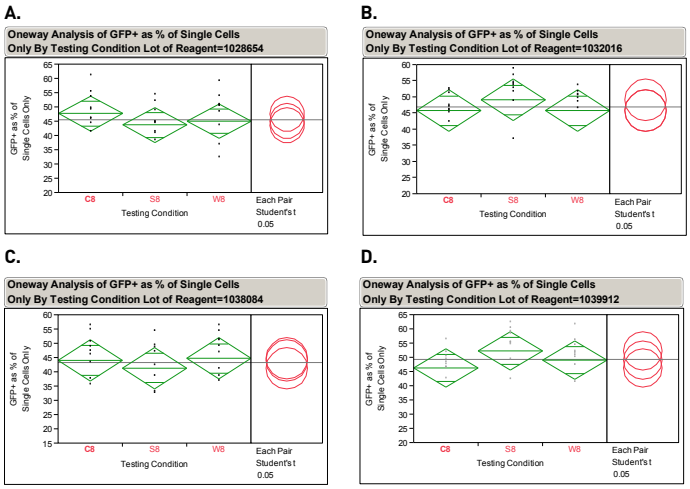

Figure 12. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent in MCF-7 (C8, control; S8, summer; W8, winter). (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084, (**D**) Lot Number 1039912.

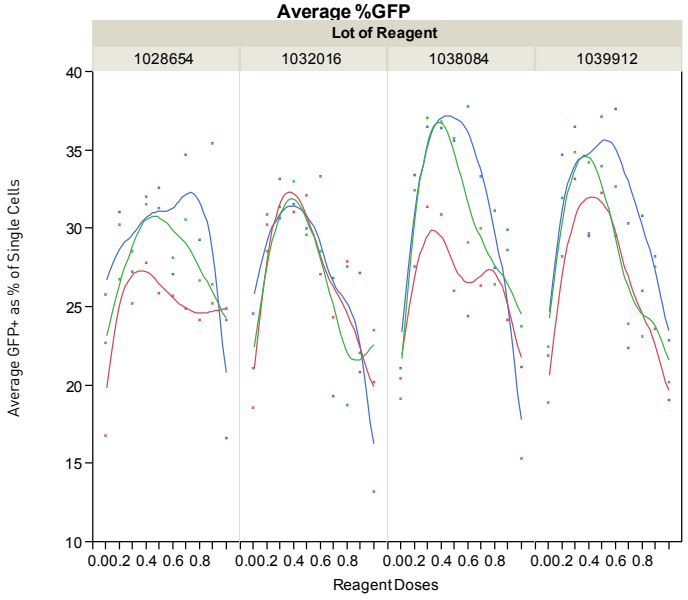

Figure 13. Titration series in Hep-G2 (average % GFP). There was no difference in transfection efficiency among summer (red), winter (green), and control (blue) samples, after summer- or winter-shipping simulation and following storage for 8 weeks at 24°C.

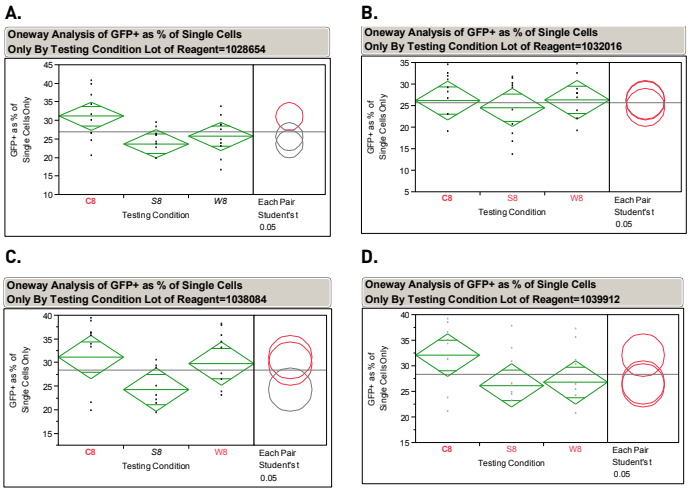

Figure 14. Statistical analysis of transfection efficiency of Lipofectamine® 2000 transfection reagent in Hep-G2 (C8, control; S8, summer; W8, winter). (**A**) Lot Number 1028654, (**B**) Lot Number 1032016, (**C**) Lot Number 1038084, (**D**) Lot Number 1039912.

The transfection efficiency of Lipofectamine® RNAiMAX reagent was measured as expression of siRNA for luciferase in the cell lines described above. The amount of siRNA was held constant while the amount of transfection reagent was titrated.

The titration series in 293-luc is shown in Figure 15. There were no significant differences among summer, winter, and control samples.

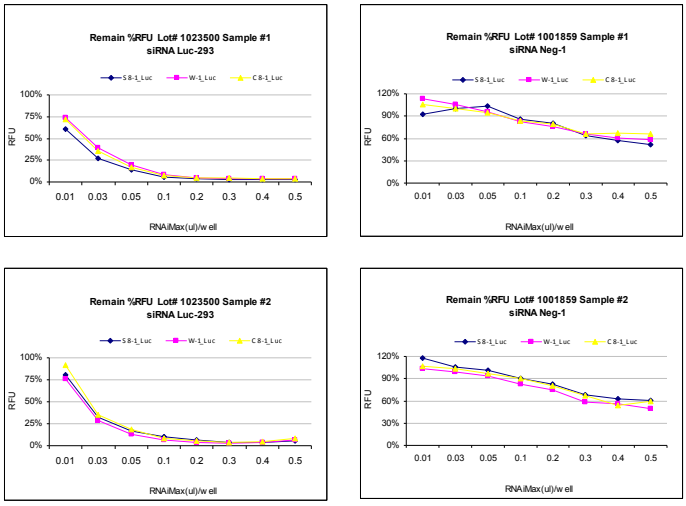

Figure 15. Transfection efficiency of Lipofectamine® RNAiMAX transfection reagent in 293-luc (black, summer; yellow, control; pink, winter).

Conclusions

The data described in this paper demonstrate that ambient shipping of Lipofectamine® transfection reagent products has no effect on their quality, integrity, and functional performance. The results of analytical and functional performance testing showed that the samples were not impacted when shipped under simulated summer or winter ambient shipping conditions.

For Lipofectamine® 2000 and Lipofectamine® RNAiMAX reagents, analytical tests showed no statistical difference in the chemical nature of the reagents when subjected to ambient shipping. Functional testing, while intrinsically variable, showed no significant differences in transfection efficiency across multiple cell lines. Accelerated stability predicted no change to shelf life stability.

The data clearly show that it is not necessary to ship Lipofectamine® 2000 and Lipofectamine® RNAiMAX reagents in EPS coolers with frozen gel packs. Because we can replace these materials, we will be shipping these products in fully recyclable corrugate containers. It must be mentioned that when orders are placed with products that must be shipped refrigerated, we may include Lipofectamine® 2000 and Lipofectamine® RNAiMAX reagents—this prevents having to ship a second container, avoiding additional material waste and greenhouse gas emissions.

By demonstrating that the quality and performance of the reagents are not compromised, we can significantly reduce the environmental impact of transport of these products. Consumption of nonrenewable raw materials will decrease by over 120 barrel equivalents of oil every year and reduce water utilization by over 30,000 liters. Our customers will see a reduction of 9,000 kg (24,000 ft^3) of EPS waste. Our planet will see a reduction of CO_2 emissions by over 80 tons every year. Finally, the packaging used to deliver the products, from the corrugate outer box to the plastic and paper containers, are fully recyclable. Please reuse the containers when possible and, when you cannot reuse, please recycle.

References

1. Anderson G, Scott M (1991) Determination of product shelf life and activation energy for five drugs of abuse. *Clin Chem* 37(3):398-402.
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†**Predicted stability was set for 6 months—the guaranteed shelf life of Lipofectamine® reagents upon receipt**

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