

Stability Testing Dharmacon™ DharmaFECT™ siRNA Transfection Reagents

Summary:

The stability of the four DharmaFECT™ Lipid Reagents was tested under six different storage conditions, including four different temperatures. Lipids were exposed to the different temperatures for a period of twelve hours (overnight) or exposed to one or three freeze cycles (simulating accidental storage of the lipids), and compared to the activity of lipids stored at the recommended temperature (4 °C). It was found that DharmaFECT siRNA Transfection Reagents are very stable and none of the tested conditions significantly affected cell toxicity or their ability to deliver siRNA efficiently under optimized conditions.

Experiment Details:

DharmaFECT 1, DharmaFECT 2, DharmaFECT 3, and DharmaFECT 4 were tested at the following conditions in order to simulate possible shipping/storage conditions as well as a more extreme high temperature (53 °C).

1. One freeze cycle at -20 °C
2. Three freeze cycles at -20 °C
3. Room Temperature overnight
4. 37 °C overnight
5. 53 °C overnight
6. Real shipping conditions: insulated box with an ice pack, left overnight.

All samples were tested for delivery efficiency (branched DNA, Panomics, Inc.) and toxicity (alarmarBlue™) in HeLa cells at the following parameters: 10,000 cells per well, 0.4 µL lipid per well of cells, 100 nM siGENOME™ Human Cyclophilin B siRNA (Cat. #D-004606-03), data taken 24 hours after transfection. All experimental samples were compared to samples treated with DharmaFECT siRNA Transfection Reagents stored at the recommended temperature of 4 °C.

Conclusion:

- None of the tested conditions significantly affected cell toxicity or their ability to deliver siRNA.
- DharmaFECT siRNA Transfection Reagents are very stable under standard delivery and storage conditions (4° C) and conditions that fall outside recommended storage.

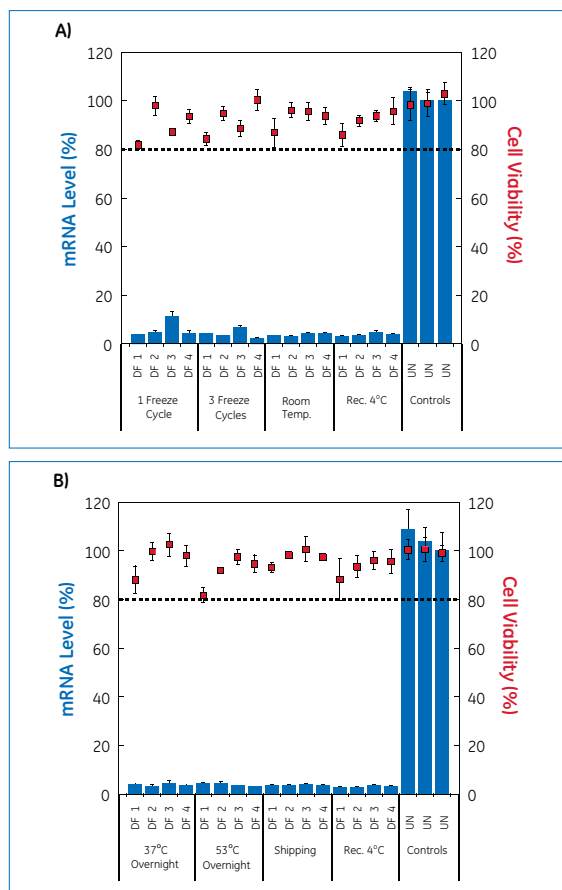


Figure 1. DharmaFECT 1, 2, 3, and 4 were tested under six different storage conditions. In Figure 1A the following three conditions were tested: one freeze cycle at -20°C, three freeze cycles at -20°C, and room temperature overnight, and compared to the recommended conditions of 4°C. In Figure 1B the following three conditions were tested: 37°C overnight, 53°C overnight, and real shipping conditions (insulated box with ice pack left overnight), and compared to the recommended conditions of 4°C. HeLa cells were transfected with 0.4 µL DharmaFECT/well and 100 nM human siGENOME Cyclophilin B siRNA (Catalog #D-004606-03) at 10,000 cells per well. Cell viability was measured with alamarBlue™ and mRNA Levels were measured with branched DNA (Panomics, Inc.) at 24 hours. The acceptable cutoff for cell viability is 80%, as denoted by the green dotted line. The experimental control samples (UN) were untransfected cells.

alarmarBlue is a trademark of Thermo Fisher Scientific, Inc. GE, imagination at work and GE monogram are trademarks of General Electric Company. Dharmacon, Inc., a General Electric company doing business as GE Healthcare. All other trademarks are the property of General Electric Company or one of its subsidiaries. ©2016 General Electric Company—All rights reserved. Version published April 2016. GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

