

Stability Testing Thermo Scientific 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.

Experimental Details

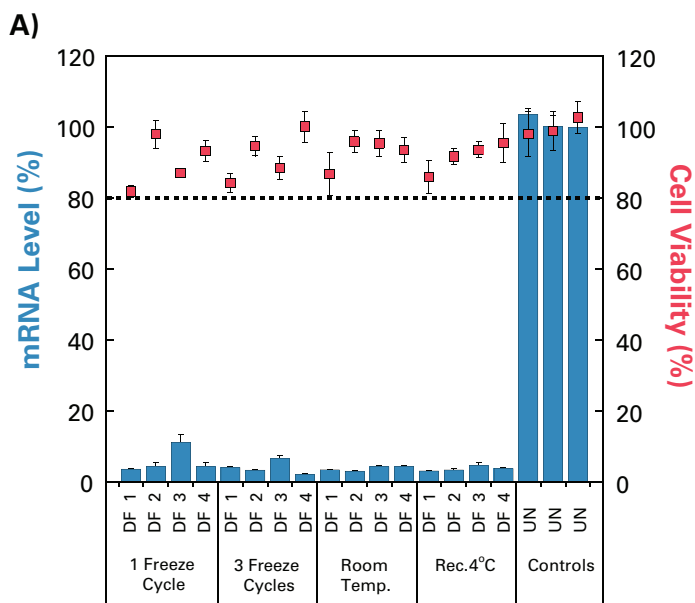
DharmaFECT 1, DharmaFECT 2, DharmaFECT 3, and DharmaFECT 4 were tested at the following conditions in order to simulate possible shipping/storage conditions as well as a more extreme high temperature (53°C).

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 (alamar Blue[™], Biosource International, Inc.) in HeLa cells at the following parameters: 10,000 cells per well, 0.4µL lipid per well of cells, 100nM siGENOME[®] Human Cyclophilin B siRNA (Catalog #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.

Conclusions

- 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.



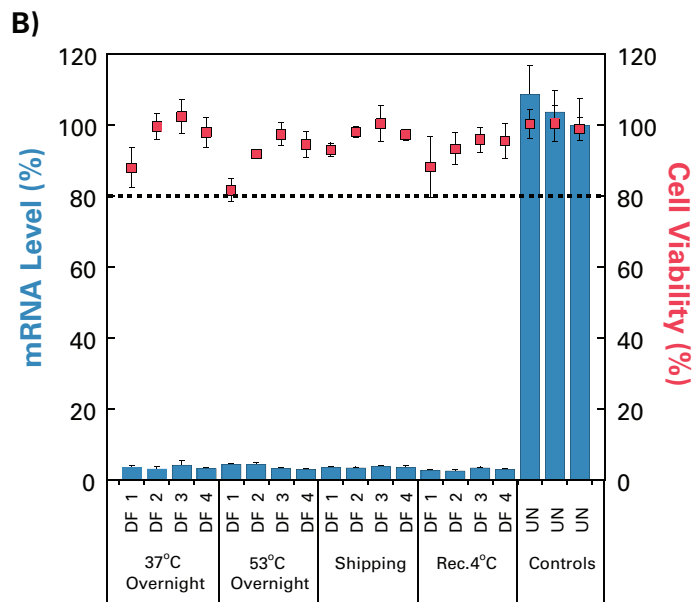


Figure 1. DharmFECT 1, 2, 3, and 4 were tested under six different storage conditions. In Figure 1A the following three conditions were tested: one freeze cycle at -20°C , three freeze cycles at -20°C , and room temperature overnight, and compared to the recommended conditions of 4°C . In Figure 1B the 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 $0.4\ \mu\text{L}$ DharmFECT/well and $100\ \text{nM}$ human siGENOME Cyclophilin B siRNA (Catalog #D-004606-03) at $10,000$ cells per well. Cell viability was measured with alamarBlue™ (Biosource International, Inc), 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

Troubleshooting

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

In North America (US, Canada, Central/South America)

Techservice.genomics@

thermofisher.com

+1 (800) 235-9880

In Europe (EU, Middle East, Africa)

Techservice.emea.genomics@thermofisher.com

(+44) 1372 840410

In Other Countries

www.thermo.com/dharmacondistributors

Literature Code: 00123-06-H-02-U

© 2010 Thermo Fisher Scientific Inc. All rights reserved. alamarBlue is a trademark of Accumed International, Inc. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.