PRODUCT BULLETIN

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Stability and shelf-life testing of Accell™ siRNA reagents

Summary

The stability of the Dharmacon™ Accell™ siRNA reagents was tested under a variety of storage conditions, including two stock concentrations, three resuspension conditions, and five temperatures. Accell siRNA was tested every three months for functionality (determined by knockdown level of the target gene) and effects on cellular viability. In addition, Accell delivery mix (1 µM working concentration of Accell siRNA in Accell delivery media) was assessed for stability when stored for up to 31 days at 4°C.

While recommended storage conditions are still highly encouraged (frozen stocks at -20°C). It was found that Accell siRNA reagents are very stable and none of the tested conditions significantly affected cell viability or the ability to silence target genes efficiently under optimized conditions.

Experimental details: Dharmacon Accell siRNA stability

The Dharmacon Accell siRNA targeting human GAPD (Dharmacon Cat# D-001930-01) and Accell Non-targeting control siRNA (Dharmacon Cat# D-001910-01) were tested at the following conditions in order to simulate standard shipping and storage conditions as well as more extreme situations that could arise.

Storage temperatures tested

1. –80°C
2. –20°C
3. 4°C
4. Room Temperature (RT)
5. 37°

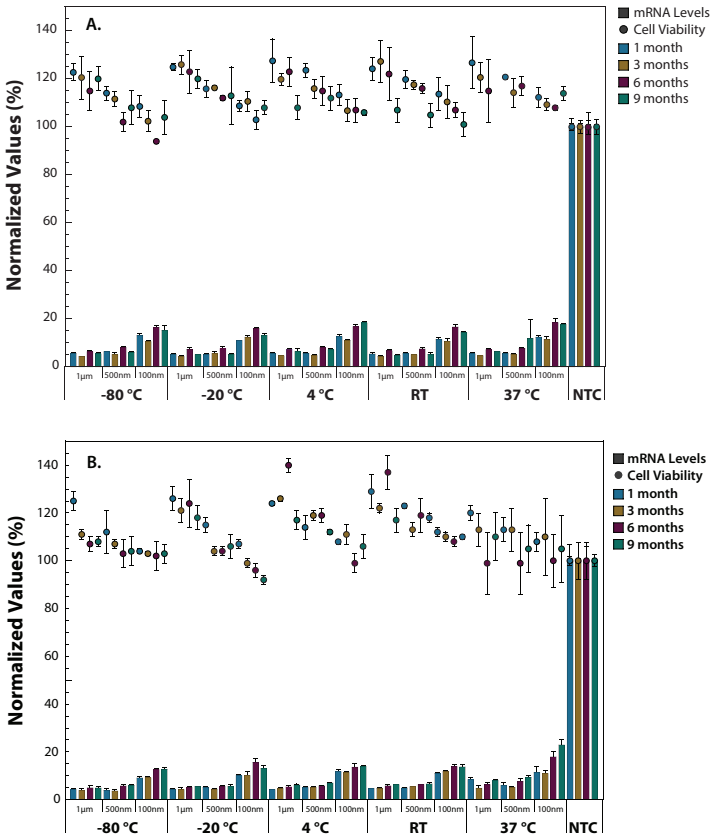

Figure 1: Accell siRNA retains functionality following storage at a variety of conditions. Accell siRNA targeting human GAPD and Accell Non-targeting control siRNA (NTC) were stored as **(A)** dry pellets and resuspended in 1x siRNA buffer to 10 µM prior to testing and **(B)** at 10 µM concentration in 1x siRNA buffer (1 mM data not shown). See text for experimental details.

Resuspension conditions tested

1. Dry pellet—reconstituted in 1x siRNA buffer to 10uM prior to testing (Figure 1a)
2. 1x siRNA buffer—1 mM and 10 µM concentration (Figure 1b)
3. RNase-free water—1 mM and 10 µM concentration (Figure 2a)
4. PBS—1 mM and 10 µM concentration (Figure 2b)

All samples were tested for silencing efficiency (Branched DNA, Panomics, Inc.) and toxicity (Thermo Scientific™ alamar Blue™) in HeLa-S3 cells at the following parameters: 10,000 cells per well, 1 µM siRNA. Data was generated 72 hours after delivery. All experimental samples were compared to samples treated with Accell delivery media alone (Cat #B-005000). Delivery media was always stored at the recommended temperature of 4°C.

Experimental details: Accell delivery mix stability

Accell siRNA targeting Cyclophilin B or Accell Non-targeting control siRNA was combined with Accell Delivery Media to a working concentration of 1 µM siRNA. This delivery mix was stored at 4°C and assessed at three siRNA doses for five time intervals within a 31 day period to determine silencing efficiency (Branched DNA, Panomics, Inc.) in HeLa cells. Accell Non-targeting control siRNA was used at 500 nM. (Figure 3)

Since Accell delivery mix is not cell type-specific, these conditions were tested to simulate experimental conditions and workflows that may require extended storage of the Accell delivery mix (starting a new screen, optimizing a new cell line, etc.).

Conclusions

- None of the tested conditions significantly affected cell viability or the ability of Accell siRNA to silence its target gene.
- Accell siRNA reagents are very stable under standard delivery and storage conditions, as well as conditions that fall outside those recommended.
- Accell delivery mix silencing performance did not decline over time and is very stable under recommended storage conditions for up to 31 days.

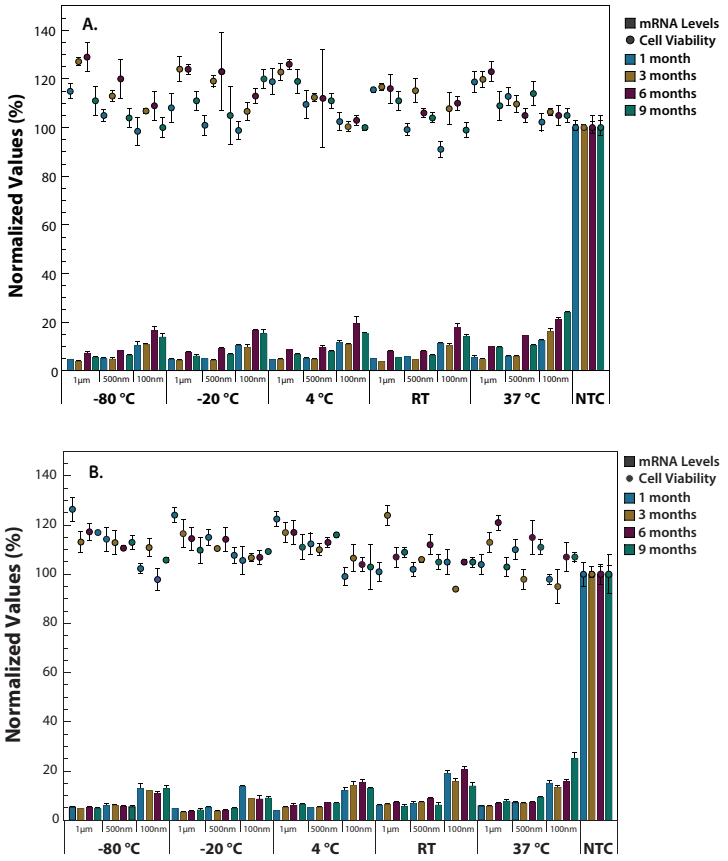

Figure 2. Accell siRNA retains functionality following storage at a variety of conditions. Accell siRNA targeting human GAPD and Accell Non-targeting control siRNA (NTC) were stored at **(A)** 1 mM concentration in RNAse-free water (10 µM data not shown) **(B)** 1 mM concentration in PBS (10 µM data not shown). See text for experimental details.

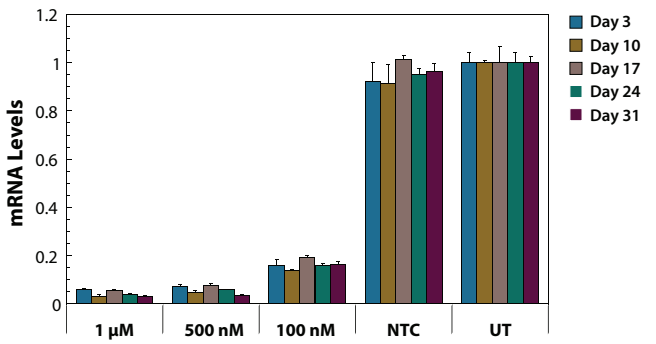

Figure 3. Accell delivery mix retains silencing performance for up to 31 days. Accell siRNA targeting Cyclophilin B and Accell Non-targeting control siRNA (NTC) were diluted to 1 µM in Accell Delivery Media. Data were normalized to untreated (UT). This delivery mix was stored at 4°C and tested for silencing performance at five time intervals to assess retention of functionality.

If you have any questions, contact

t +44 (0) 1223 976 000 (UK) **or** +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f + 44 (0)1223 655 581
w horizondiscovery.com/contact-us **or** dharmacon.horizondiscovery.com/service-and-support
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

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