Performing a Normalization Assay with the Thermo Scientific Multidrop Combi nL Reagent Dispenser and FILLit Software

Diane Baraket, Applications Support Specialist,Thermo Fisher Scientific, USA

Technical Note

Goal

This technical note offers instructions on how to set up the Thermo Scientific™ FILLit™ software to run a normalization assay with the Thermo Scientific™ Multidrop™ Combi nL low volume reagent dispenser. Normalization of nucleic acid concentrations is used in DNA sequencing, qPCR and NGS (Next Generation Sequencing).

Introduction

Purified nucleic acids are used as templates for several molecular biology methods, e.g. qPCR and DNA sequencing. Typically DNA or RNA samples have variable concentration after purification, but in many cases the same specific concentration is required for all templates in the assay. Normalization of concentrations performed manually is tedious, especially when working with a high number of samples. Addition of various volumes of diluents on each template is time-consuming, and when working in small volumes, this produces many challenges to meet the requirements needed for accurate and consistent dispensing.

Normalization of nucleic acid concentrations is commonly used in applications that require DNA Sequencing, qPCR and NGS. Production of quality DNA sequence data in a consistent and cost-effective manner requires a straightforward process for balancing template concentrations. In high-throughput NGS, equimolar amounts of each nucleic acid samples are to be prepared in order to pool libraries.

Normalization can also be used in preparing source plates for storage. For example, after dispensing compounds, Dimethyl sulfoxide (DMSO) is dispensed to obtain equal total volume in each well.

Multidrop Combi nL dispenser and FILLit software

A low volume reagent dispenser, like the Multidrop Combi nL, is an excellent choice to automate the normalization process. Multidrop Combi nL utilizes a pressurized reagent bottle and individually controlled solenoid valves. This technology offers reliable low volume dispensing. This feature also allows a different volume to be dispensed into each well.

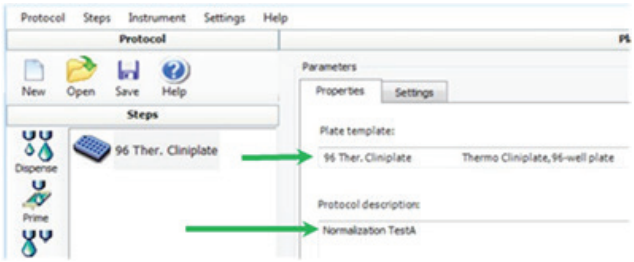
FILLit software allows copying/pasting volumes directly from a Microsoft® Excel® worksheet to the dispense step layout in the FILLit software.

The features of the Multidrop Combi nL dispenser and FILLit software simplify the performance of the normalization assay, while increasing accuracy.

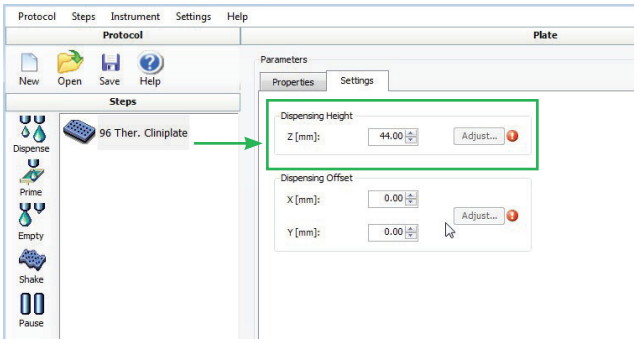
Thermo SCIENTIFIC

Setting up a normalization protocol with the FILLit software

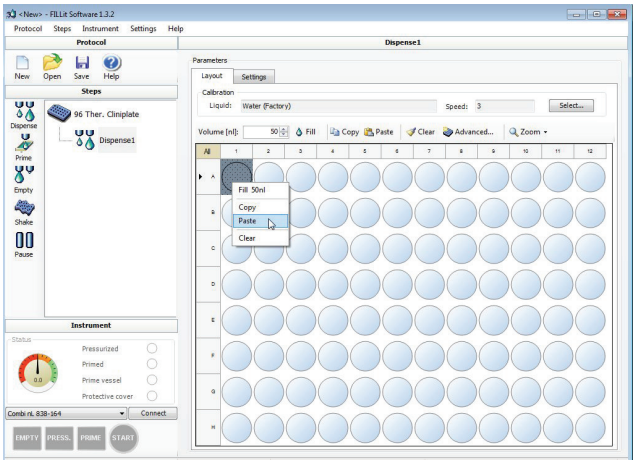
1. In the FILLit software, under Parameters within the Properties tab, select the plate. Enter the description of the protocol.



2. Optional step:
Under the Settings tab, change height accordingly based on plate/tube/rack total height (Max 55mm).



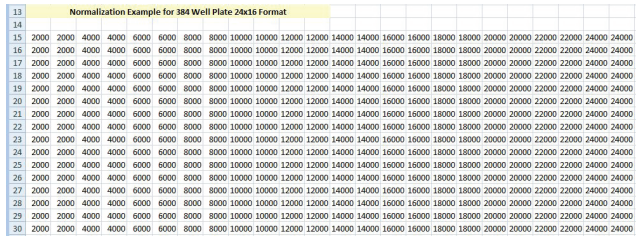
3. Select a Dispense step by clicking on the Dispense icon from the list of available steps on the left.

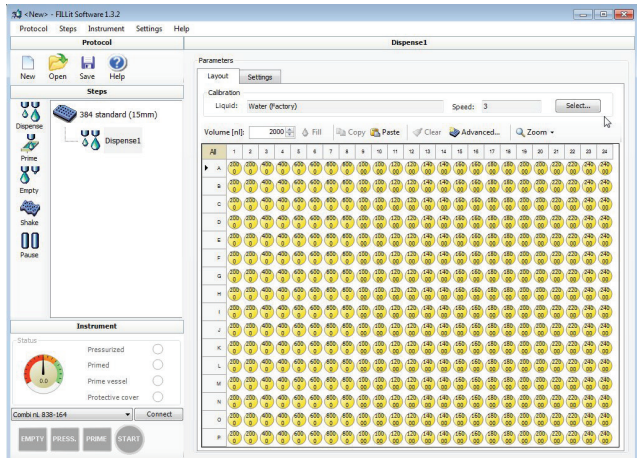


4. Have your volumes ready to select from an Excel work sheet. Copy data. Click the Paste button in the Layout tab; this automatically prefills the selected volume data into the plate layout in the FILLit software.

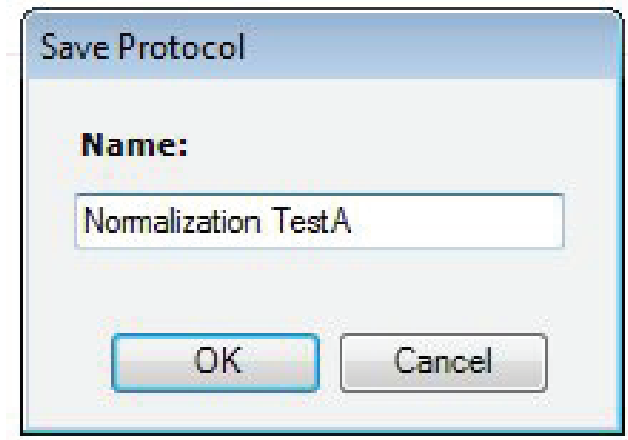
Note: *the Excel sheet should be in a grid to match the plate layout as follows:*

1. *12 x 8 grid = 96 well plate*
2. *24 x 16 grid = 384 well plate*

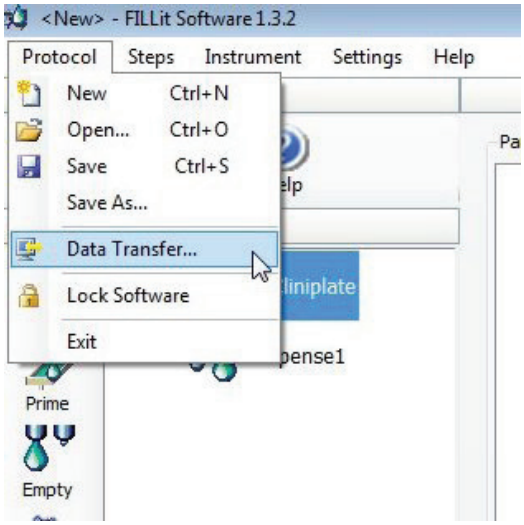




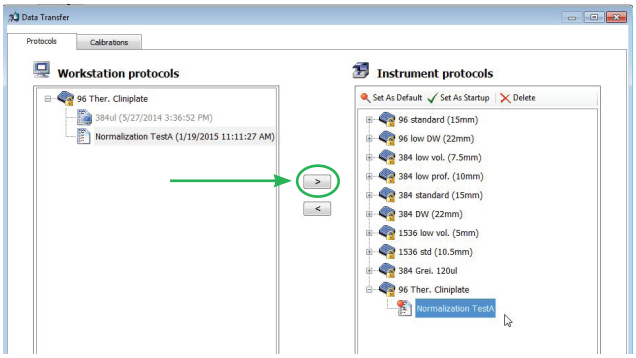
5. Under the Protocol menu, save the protocol with a name.



6. Go to Protocol menu, select Data Transfer, highlight the protocol to transfer to onboard software (it is under the corresponding plate name).



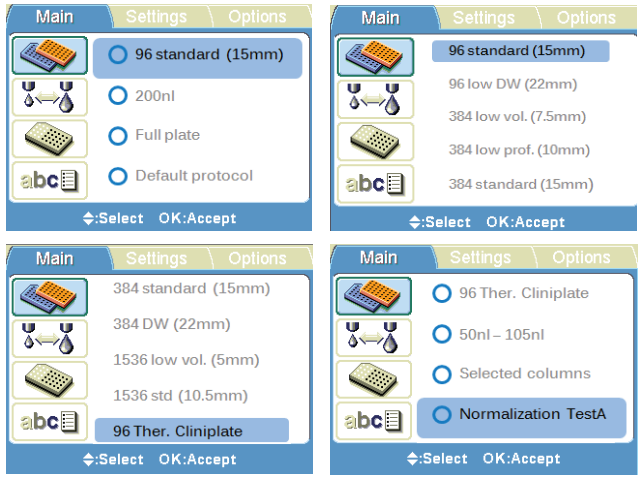
7. Click the arrow icon pointing to the right. Click yes to the next question (asking about calibration with water). The protocol is shown in the Instrument Protocol list under the corresponding plate type.



8. Close the window.

9. Disconnect instrument from FILLit software.

10. Go to the onboard software (MAIN tab), select the corresponding plate, scroll through to the list of protocols and select the protocol. Press OK.



11. Press START

Summary

The copy/paste feature in the FILLit software is a handy tool which allows simple transfer of volumes from an Excel spreadsheet into the FILLit software. Dispensing technology used in the Multidrop Combi nL makes it possible to dispense different volumes into each well of the plate which is needed to run a normalization assay.

www.thermoscientific.com/multidrop

North America:
USA/Canada +1 800 625 4327

Europe:
Austria +43 1 801 40 0
Belgium +32 2 482 30 30
France +33 2 28 03 20 00
Germany National Toll Free 08001-536 376

Germany International +49 6184 90 6940
Italy +39 02 95059 1
Netherlands +31 76 571 4440
Nordic/Baltic/CIS countries +358 10 329 2200
Russia +7 (812) 703 42 15
Spain/Portugal +34 93 223 3154
Switzerland +41 44 454 12 12
UK/Ireland +44 870 609 9203

Asia:
India +91 22 5542 9494
Japan +81 45 453 9220
China +86 21 6865 4588 or +86 10 5850 3588
Other Asian countries +852 2885 4613
Countries not listed: +49 6184 90 6940
or +33 2 28 03 20 00

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

TN_MDCombinL_Normalization_0116