

Dispensing

Performing a normalization assay with the Multidrop Combi nL Reagent Dispenser and FILLit Software

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 Reagent Dispenser. Normalization of nucleic acid concentrations is performed for DNA sequencing, qPCR, and next-generation sequencing (NGS).

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 concentrations after purification, but in many cases the same specific concentration is required for all templates in the assay. Manual normalization of concentrations is tedious, especially when working with many samples. The addition of various volumes of diluents to each template is time-consuming, and when working with small volumes, it is challenging to meet the requirements needed for accurate and consistent dispensing.

Normalization of nucleic acid concentrations is commonly performed in applications that require DNA sequencing, qPCR, or NGS. Production of high-quality DNA sequencing data in a consistent, cost-effective manner requires a straightforward process for standardizing template concentrations. In high-throughput NGS, equimolar amounts of each nucleic acid sample need to be prepared in order to pool libraries.

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



Multidrop Combi nL dispenser and FILLit Software

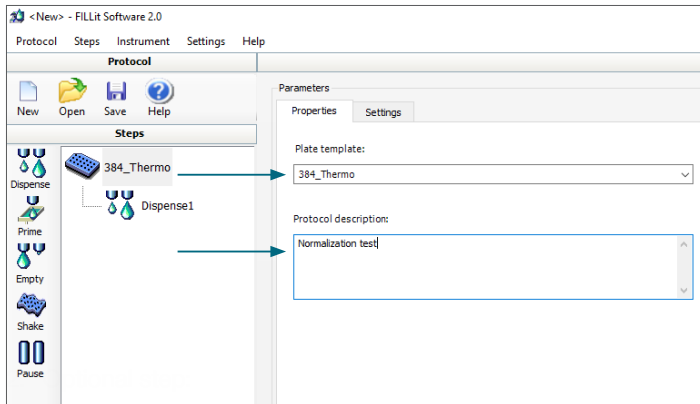
A low-volume reagent dispenser, such as the Multidrop Combi nL dispenser, is an excellent choice to automate the normalization process. The Multidrop Combi nL Reagent Dispenser uses a pressurized reagent bottle and individually controlled solenoid valves. This technology offers reliable low-volume dispensing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

The FILLit Software enables copying and pasting volumes directly from a Microsoft™ Excel™ worksheet to the dispense step layout in the FILLit Software.

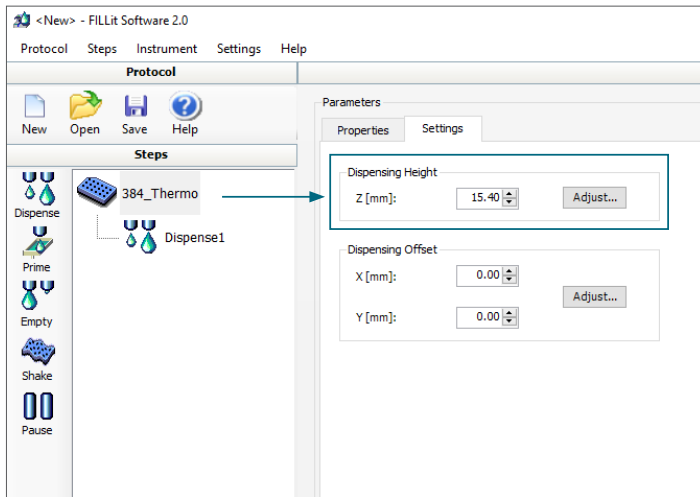
The features of the Multidrop Combi nL Reagent Dispenser and the FILLit Software simplify the performance of the normalization assay, while increasing accuracy.

Setting up a normalization protocol in the FILLit Software

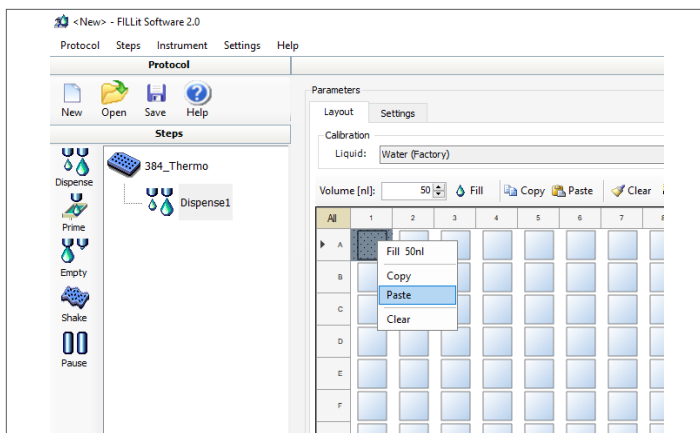
1. In the FILLit Software, under “Parameters” and within the “Properties” tab, select the “Plate template”. Enter the description of the protocol.



2. Optional step: Under the “Settings” tab, change the height based on plate/tube/rack total height (maximum of 55 mm).



3. Select a “Dispense1” step by clicking on the “Dispense” icon from the list of available steps on the left.



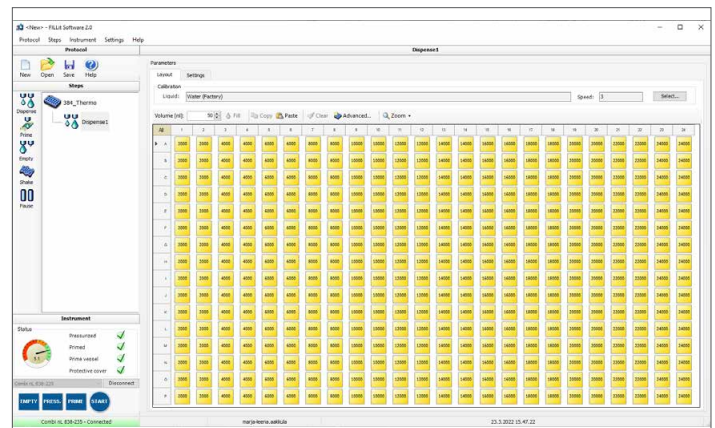
4. Have your volumes ready to select from an Excel worksheet. Copy the data from the Excel file. Click the “A1” well in the FILLit Software “Layout” tab and click the “Paste” button; this automatically prefills the selected volume data into the plate layout in the FILLit Software.

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

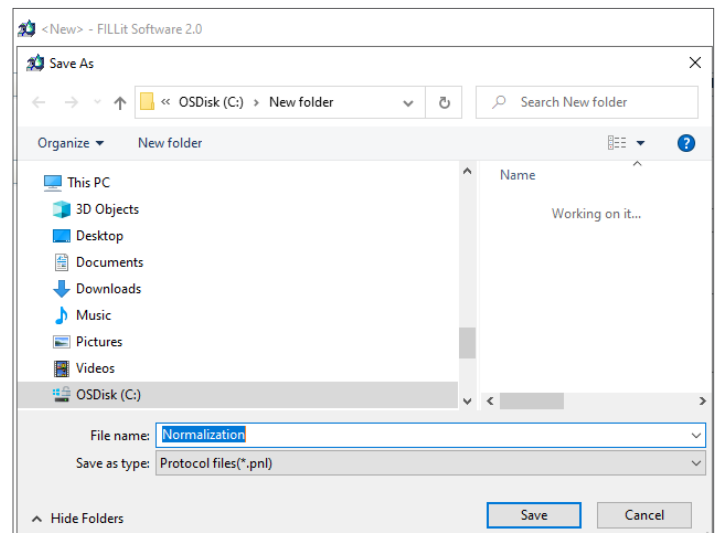
- 12 x 8 grid = 96-well plate
- 24 x 16 grid = 384-well plate

Normalization example for a 384-well plate in 24 x 16 format*									
	1	2	3	4	5	6	7	8	9
A	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
B	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
C	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
D	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
E	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
F	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
G	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
H	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
I	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
J	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
K	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
L	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
M	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
N	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
O	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000
P	2,000	2,000	4,000	4,000	6,000	6,000	8,000	8,000	10,000

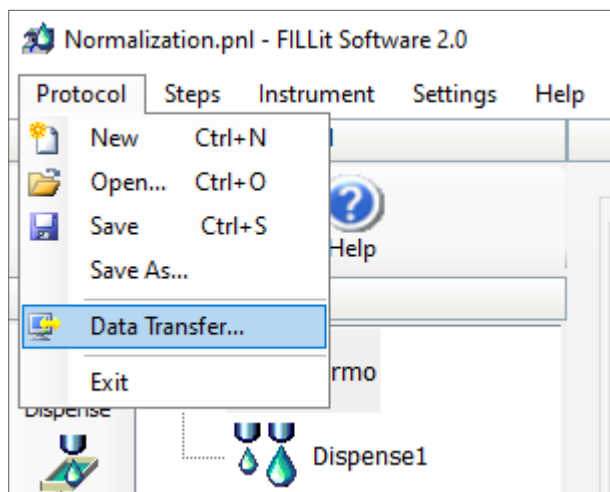
* Only part of the 384-well plate is shown in the example.



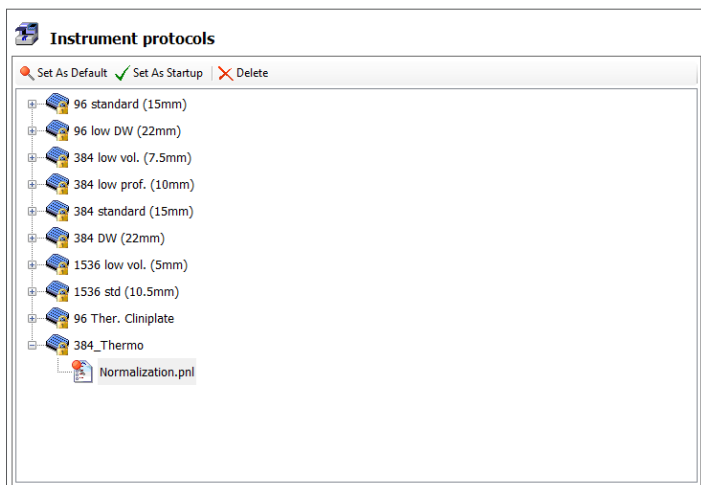
5. Under the “Protocol” menu, save the protocol with a name.



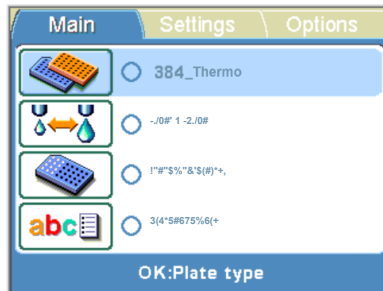
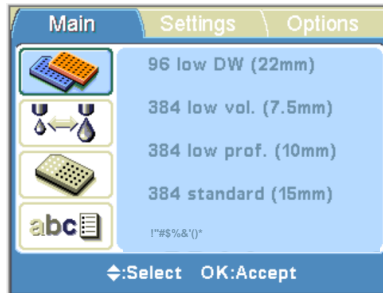
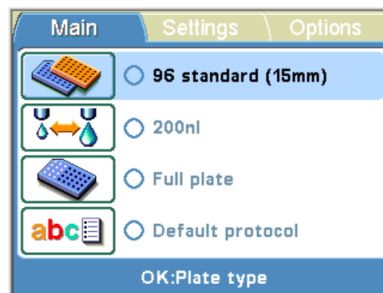
- Go to the “Protocol” menu, select “Data Transfer”.
- Select the “Protocol” tab. Click “Upload” to transfer a protocol to the instrument. The “Windows File Open” dialog box opens.



Select the protocol file (*.pnl). Click “Yes” for the next question about calibration with water. The protocol is shown in the “Instrument protocols” list under the corresponding plate type.



- Close the window.
- Disconnect the instrument from the FILLit Software.
- Go to the onboard software (“Main” tab) and scroll through to the plate list to select the corresponding plate. Go to “abc” and scroll through the list of protocols to select the protocol.



- Select “Start”.

Summary

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