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

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.



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

Protocol Steps Instrument Settings Protocol	Help
🗅 🎅 📙 🔘	Parameters
New Open Save Help	Properties Settings
Steps	
Dispense 96 Ther. Cliniplate	Plate template: 96 Ther. Cliniplate Thermo Cliniplate, 96-well plate
	Protocol description:
84	Normalization TestA

2. Optional step:

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

Protocol	Plate
📄 📂 🛃 🥑 New Open Save Help	Parameters Properties Settings
Steps	
Vispense 96 Ther. Cliniplate	Z [mm]: 44.00 Adjust
No. 1	Dispensing Offset
Prime	X [mm]: 0.00 🛬
Empty	Y [mm]: 0.00 🚔
Shake	
00	
Pause	

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

Protocol	Dispense1	
	Devender	
🔟 📂 🖬 🔮	award Cattions	
Steps	Calculation	
U 🚓	Liquid: Water (Factory) Speed: 3 Select	
96 Ther. Cliniplate		
U Dimansal	Volume [nl]: 50 🔄 🍐 Fill 🦾 Copy 😤 Paste 🚿 Clear 📚 Advanced 🔍 Zoom 👻	
o o o ouperiori	Al 1 2 3 4 5 5 7 8 9 13 11	12
de de CO		
Instrument		
Pressurized O Primed O		\bigcirc
Prime vessel		

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

13		N	ormalia	ation	Examp	le for 3	84 We	II Plat	e 24x1	6 Form	at														
14																									
15	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
16	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
17	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
18	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
19	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
20	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
21	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
22	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
23	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
24	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
25	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
26	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
27	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
28	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
29	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	
30	2000	2000	4000	4000	6000	6000	8000	8000	10000	10000	12000	12000	14000	14000	16000	16000	18000	18000	20000	20000	22000	22000	24000	24000	

	Protocol	1										Die	anre	.1											
-		12013										UB	Jensy												
	📂 🖬 🕐	Paramet	ers .																						
New	Open Save Help	Layor	Jt _	Set	ings																			_	_
	Calb	Calibration																		Colum					
00	-	Liquid: Water (Pactory)											spee	0:	3				Juletter			- N			
ispense	UU	Volun	ne (nl)	: 2000 🔄 👌 Fill 🗈 Copy 📸 Paste 🛷 Clear										er i	Advanced					Q Zoom +					
44	Dispense1	Al	1	2	3	4	5 6	7	8		10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Prime		• A	200	250	430	400	200 600	0	0	100	100	-	120	(#)	1	170	160	180	180	200	200	220	220	-	4
<u>8</u> *			-	250	400	400	20 600	.00	.00	100	100	120	-	(40)	(10)	150	160	180	180	-	100	220	220	240	24
Empty			100	100	400	400	0 0	.00	0	100	100	00	00	(B)	(B)	100	100	180	180			220	225	00 000	00
~		-	100	-	400	400.4		0	0	00	00	00	00	8	(B)	100	460	480	100	00	00	00	00	00 5%	00
Shake		0		0	0	0	00	0	0	00	00	02	02					00	00	00	00	00	00	02	0
UU		-		0	0	0	0	C	0	00	600	00		0	0				00	00		00	00	00	6
Pause		F	٢	٢	0	0		C	0				3	(100					3	6
		a	٢	٢	400	*	000	0	0	0	**	8	٢	۲	۲	8	360	180	180	8	**	8		8	64
		н	٢	٢	400	***	000	000	0			8	-	۲	۲	60	160	(IED	180	-		8		3	3
			300	(50)	450	450	0 600	0	0	100	100	(13) (13)	33	-	(#) (*)	(50)	(150	1ED	180			230	230	340 00	240
	Instrument	J	(350	(250)	400	450	600 600	(600	000	100	100		120	(-	(150	160	(IED	180	200		220	(23)	240	24
itatus —	Pressurized	ĸ	250	(200	400	400	500 600	600	(600	100	100		(23)	(10) (10)		(50)	160	180	180	200	200	220	(220)	240	240
1	Primed O	L	250	250	400	400	500 600	600	600	100	100	-23			-	150	160	150	180	200	200	20	223	540	2542
	Prime vessel		250	250	400	400	500 600	600	600	100	100	120	120	540	540	150	160	180	180	200	200	220	220	240	/241
	Protective cover	-	200	200	400	400 4	600	600	.00	100	100	120	120	00 (540	00 (40)	260	160	150	180	200	200	220	220	240	24
mbi nL 8	338-164 V Connect		0	350	410	400	0 0	600	60	00	00	00	00	00	00	00	450	180	180	200	00	00	220	00	00
-		0	0	0	0	0	00	0	0	00	00	02	02	00	00	00	00	00	00	00	00	00	00	02	00

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

Normalization TestA	

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

Pro	tocol Steps Instrument Settings	Help
	New Ctrl+N Open Ctrl+O Save Ctrl+S Save As	Pa
P	Data Transfer Lock Software	
Prin 8 Emp	pense1	

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.
- 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

© 2016 Thermo Fisher Scientific Inc. All rights reserved. Microsoft and Excel are registered trademarks of Microsoft Corporation. All other trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific Inc. products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

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

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

Asia:

