Tech Note

Guidelines for the Provision of Residual Volumes of Assay Buffer in Microplate Wash Assays

Setting aspiration manifold z-heights in ELx405™ / EL406™ software to provide predetermined residual volumes in the final dispense/aspirate step of a microplate wash cycle

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Z-height of the aspiration manifold can be used to leave behind accurate and precise residual volumes of assay buffer in the final dispense/aspirate step of a wash cycle. The properties of the assay buffers, such as ionic strength and the use of detergents can have a significant impact on the residual volumes, however. Here we provide guidelines for gauging residual volumes for a number of assay buffers and microplate types.

Introduction

Wash assays are indispensable formats for the selective and sensitive quantification of countless analytes. Wash steps provide the means for removal of interfering components in the sample matrix and excess added reagent. ELx405™ is the industry standard microplate washer used in many applications including ELISA[1], Mesoscale MSD assays[2], radiometric uptake assays[3], FLIPR assays using fluorescent Ca^{2+} flux dyes[3,4] and immunocytochemistry[5].

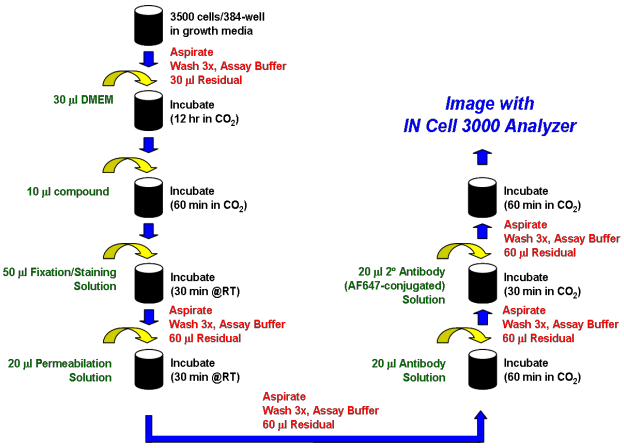


Figure 1. Workflow for Gluocokinase Translocation Assay. Green text indicates assay component addition to microplate; red text indicates operations performed by the ELx405™; black text shows incubation conditions; and blue text informs on imaging reader used: GE Healthcare's IN Cell 3000 Analyzer.

Many of these applications require that a predetermined residual volume be left in each well following the final dispense/aspiration step of the wash cycle. As an example, the following workflow used for an immunocytometric assay to determine the activation and translocation of the protein glucokinase in rat primary hepatocytes involves numerous steps requiring the leaving of residual volumes, in some cases, of different volumes[5].

The popularity of ELx405 stems from a set of unique features that provide excellent wash speed, efficiency and reproducibility. Key to this is the patented Dual-Action™ manifold that allows for overflow and well bottom washing and important to this exercise, independent control of aspiration and dispense manifolds in three dimensional space (see Figure 2) that allows for the accurate and precise provision of residual volumes of assay buffer at the end of a wash cycle.

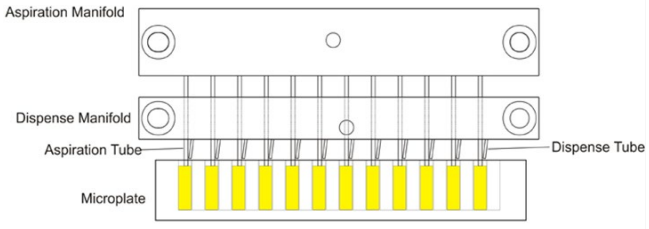
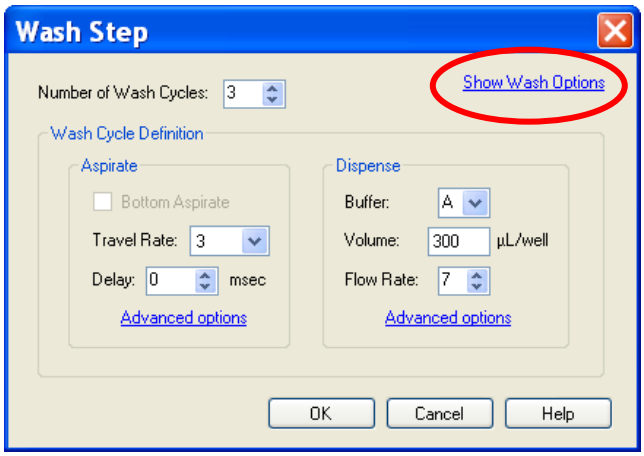


Figure 2. Dual-Action™ manifold of ELx405™ Microplate Washer.

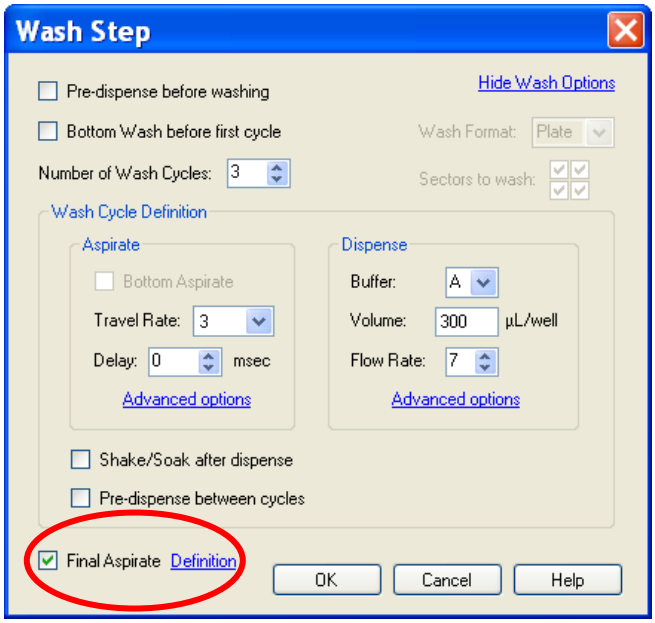
A number of factors can affect the volume of assay buffer left behind after the last aspirate / dispense step of the wash cycle in addition to the obvious parameters such as z-height of the aspiration manifold and well density / well volume of the microplate used. These include the ionic strength of the assay buffer and various additives, such as detergents, the composition of the microplate (polystyrene, polypropylene, etc.) and any microplate coatings used that can affect surface tension of the fluid. In this study, we will provide guidelines for the provision of residual volumes based on aspiration manifold z-height for a number of assay buffers differing in ionic strength and with and without the use of Tween 20 for Corning 96- (untreated and tissue culture-treated) and 384-well (untreated) densities. Also some common buffers used for cell-based assays, such as Tyrode's buffer and DMEM-F12, will be tested. Testing was performed with the EL406™ Microplate Washer Dispenser, but findings will be equivalent for ELx405™.

Methods – LHC™ Software Control

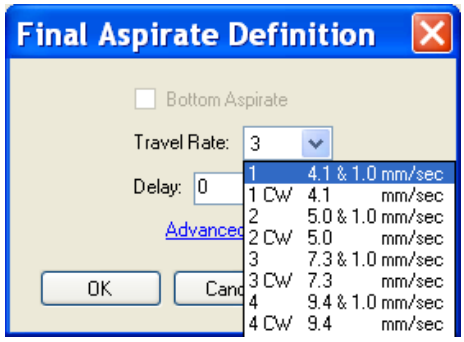
For the residual volume testing described below the Liquid Handling Control™ (LHC™) Software was used to program the wash protocol (note: these values can also be programmed via the keypad by selecting **Wash>Options**). This option is defined in the wash step by selecting Show Wash Options:



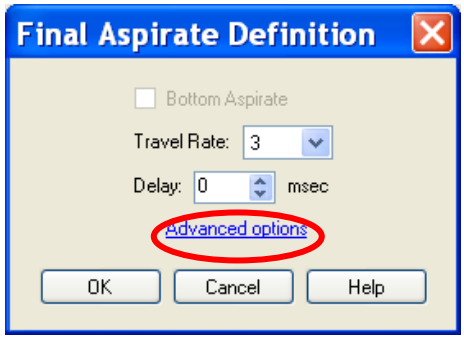
Once selected, the screen will expand to include "Final Aspirate Definition":



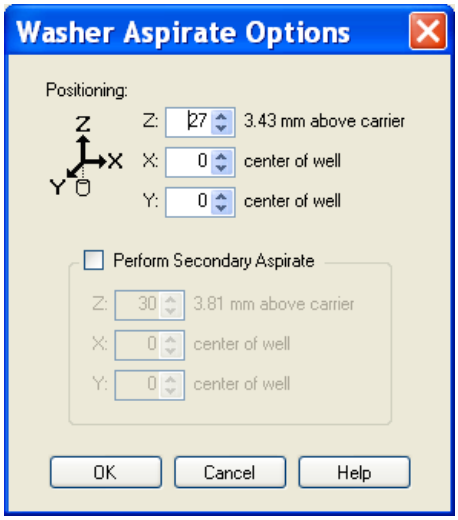
By checking the options box and clicking on Definition, the parameters of the final aspiration can be set independently of other aspiration step in a multi-step wash protocol. Here one can define the Travel Rate between settings 1-5 for non-cell based assays or 1-4CW or 6CW for cell-based assays (CW) and delay time at bottom of well during the aspirate step:



To define the Z, X and Y positions select Advanced options:



The positions are defined in motor steps, although values entered are immediately converted to units of measure (mm). Therefore, it is possible to adjust these values in regards to well position if the plate geometry is known. Furthermore, by calculating the cylindrical volume one can estimate a starting point for the Z-height position remembering that the well bottom may be up to several mm above the carrier.



The experimental values shown in the tables in the Results section were determined by making incremental changes to the Z-height offset *only* to help determine a representative range of values for a sample of plate formats and types. Due to the diverse needs of various experimental protocols each solution/plate combination will require additional empirical testing to determine the optimal settings for a particular residual volume.

Residual volume testing was performed to determine the average residual volume per well and standard deviation expressed in units of volume (µL) at a 95% confidence interval. All measurements included performing the wash protocol in triplicate for each plate type at each Z-height offset indicated in each of the tables. The average residual volume per well was calculated using the mass of the residual volume and density of the solution being tested. Each solution included FD & C #1 blue dye to obtain a final OD 450 nm between 0.1 and 1.0 using dual-wavelength measurement (630 nm - 450 nm). Residual volume per well was calculated based on the absorbance measurement data using a residual factor (mean OD450/avg mass per well) * OD. Residual volume per well was then used to calculate standard deviation and 95% confidence intervals (Microsoft Excel).

Results and Discussion

Corning, Flat Bottom 96-well Microplate (p/n 9017)

For 96-well microplates, aspiration manifold z-heights reflecting residual volumes in the range of 25 µL to 100 µL (~ 8% < well volume < 33%) were investigated. Volumes lower than 25 µL were not investigated as these tended to provide incomplete coverage of well bottom surface due to surface tension.

Effect of Buffer Concentration

The effect of buffer concentration on residual volumes is demonstrated in Figure 3. It is apparent that there is a significant difference in residual volume obtained for like z-height settings when the HEPES buffer concentration is increased to 100 mM.

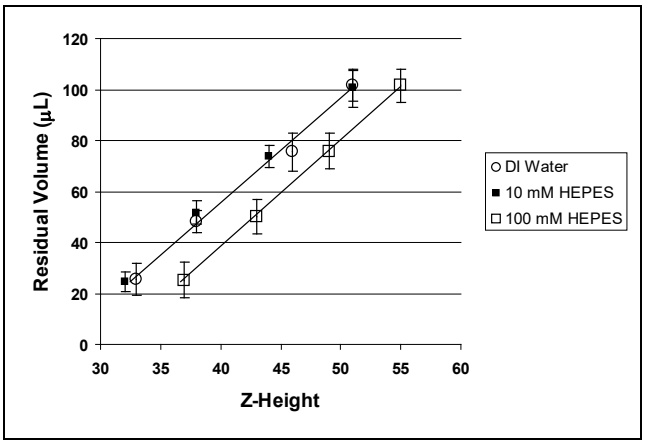


Figure 3. Effect of HEPES buffer concentration on residual volumes as a function of z-height of the aspiration manifold.

For a given z-height, there is approximately 20 µL less residual volume left behind using 100 mM HEPES relative to 10 mM HEPES or de-ionized water. At a 95% confidence level, there is no difference in residual volumes for a given z-height between 10 mM HEPES and de-ionized water.

Effect of Detergent – Tween 20

Detergents act to lower surface tension of fluids which can impact z-height control of residual volumes. Figure 4 demonstrates the effect of 0.1% and 1.0% Tween 20 on residual volumes as a function of aspiration manifold z-height.

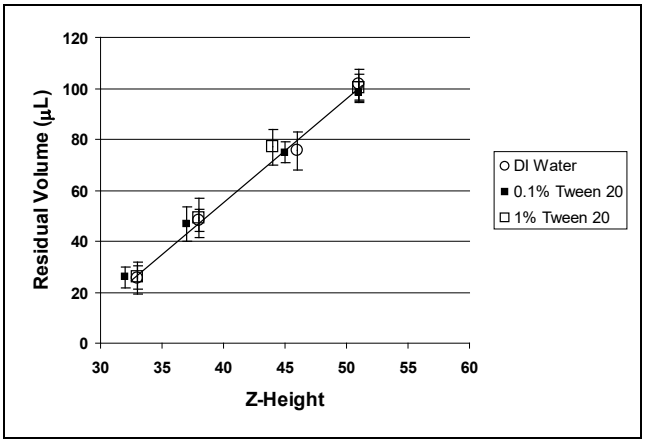


Figure 4. Effect of Tween 20 concentration on residual volumes as a function of z-height of the aspiration manifold.

At a 95% confidence level, there is no difference between the use of de-ionized water, 0.1% Tween 20 and 1.0% Tween 20.

Use of Common Cell-based Assay Buffers

Cell-based assays often use media such as DMEM-F12 which contains amino acids and glucose as well as salts to buffer and create an isotonic environment for the cells. DMEM is typically used while plating cells and can also be used during cell stimulation when receptor agonists are added in conjunction. Addition of detection reagents often uses a common buffer such as Tyrode's buffer. While the buffer is devoid of amino acids, the solution is isotonic with cells and does usually contain glucose. Figure 5 portrays residual volumes as a function of aspiration manifold z-height for these two common buffers in comparison to de-ionized water.

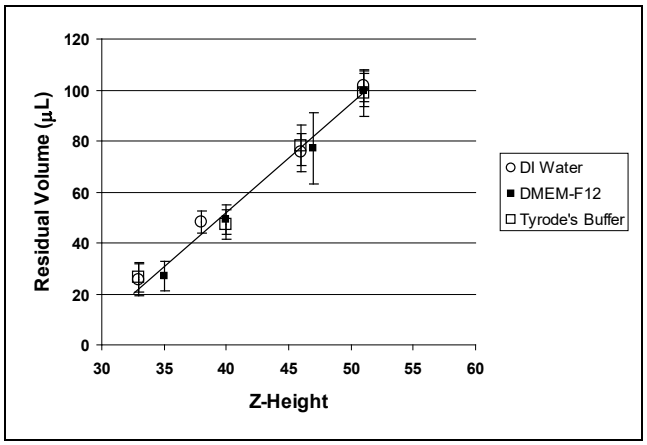


Figure 5. Effect of common cell-based assay buffers on residual volumes as a function of z-height of the aspiration manifold

Surprisingly, there is no significant difference in residual volume for a given z-height between these high ionic strength assay buffers and de-ionized water at the 95% confidence level. Presumably, there are other parameters than ionic strength at play here that makes for different performance relative to 100 mM HEPES. For example, Tyrode's buffer contains 1% bovine serum albumin, which can influence the residual volume – z-height relationship.

Effect of Tissue Culture Treatment of Microplates

Often, cell-based microplate assays use tissue culture-treated polystyrene microplates that have been specially treated to be sterile and allow for good cell adherence. Tissue culture treatment of a microplate surface is typically applied in a plasma oven. The tissue culture treatment cross-links carboxyl and amine groups with the correct net charge. This attracts mammalian cells and promotes good adherence necessary for satisfactory assay performance. Figure 6 examines the effect of tissue culture treatment on a 96-well flat bottomed microplate using Corning's tissue culture-treated plate (p/n 3603) and the HEPES buffers.

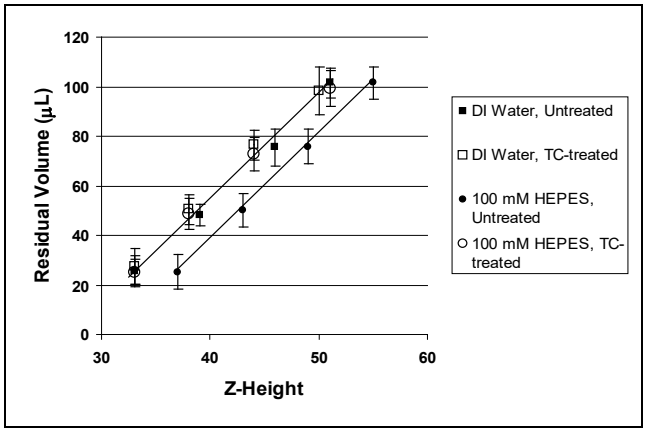


Figure 6. Effect of using tissue culture-treated microplates on residual volumes as a function of z-height of the aspiration manifold.

It is apparent that tissue culture-treatment of polystyrene flat bottomed plates negates the effects of buffer concentration seen earlier. From data available in the appendices, it is apparent that all fluids and buffers tested provided equivalent residual volumes for a given aspiration manifold z-height. This suggests that tissue culture-treated microplates should be used for accurate provision of residual volumes without influence from assay buffer additives.

Corning, Flat Bottom, 384-well Microplate (p/n 3702)

For 384-well microplates, aspiration manifold z-heights were investigated that provided residual volumes ranging from 5 µL to 25 µL (~ 6% < well volume < 30%). It was suspected that the much lower surface area of the 384-well plate compared to the aspirate pin size may negate any solution effects seen with the untreated 96-well microplate. Thus experiments were performed with an untreated microplate. Figure 7 demonstrates that there is no statistically relevant effect on residual volumes as a function of aspiration manifold z-height for increasing buffer concentration or use of detergent.

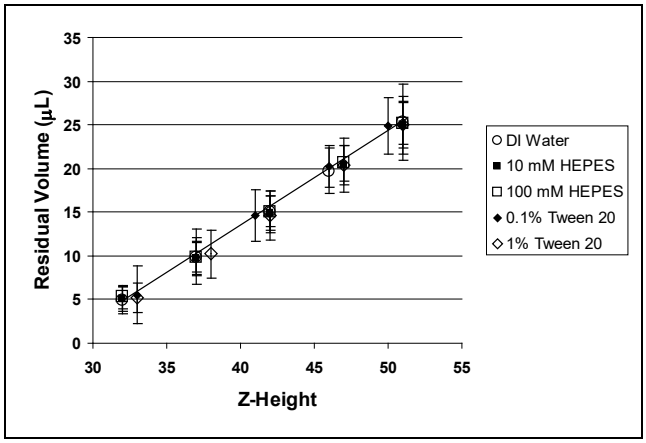


Figure 7. Effect of HEPES buffer concentration on residual volumes as a function of z-height of the aspiration manifold.

Conclusions

Tissue culture-treated microplates appear to be the best option for the most accurate provision of residual volumes. This is a fortunate circumstance as most applications requiring residual volumes being left at the end of a wash cycle involve cell-based assays which are particularly suited to tissue culture-treated plates. The table below provides some guidelines for the selection of aspiration manifold z-height to provide certain residual volumes in both 96- and 384-well Corning, flat bottomed, tissue culture-treated microplates. In the case of 384-well microplates, tissue culture-treated microplates are not required to reduce buffer effects on residual volumes, but for cell-based assays, it is still recommended to use the tissue culture-treated options.

	Aspiration Manifold Z-Height	
Residual Volume (µL)	**96-well**	**384-well**
5		**33**
10		**37**
15		**42**
20		**47**
25	**33**	**51**
50	**38**	
75	**45**	
100	**51**	

Table 1. Guidelines for achieving residual volumes in tissue culture-treated plates based on aspiration manifold z-height settings. Note: settings may vary somewhat dependent on different manufacturer's microplates and the use of different assay buffers not tested here.

References

1. L. Turunen et al. (2009) Journal of Biomolecular Screening, 14(3), pp. 282-293.
2. Y. Lu et al. (2007) Current opinion in Pharmacology, 7(5), pp.541-546.
3. R. Wagstaff et al. (2007) Journal of Biomolecular Screening, 12(3), pp. 436-441.
4. Agilent BioCel System Configuration: Automated FLIPR Assay in 384 Format – Application Bulletin, available www.agilent.com/lifesciences/automation
5. M. Wolff et al. (2008) Journal of Biomolecular Screening, 13(9), pp. 837-846.

Appendices – Tables of Original Data

96-well flat bottomed Corning Costar PN9017														
	DI Water		10 mM HEPES		100 mM HEPES		0.1% Tween 20		1% Tween 20		Delbecco's Media		Tyrode's Salt Solution	
Z Position	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL
23														
26	11.390	2.357	13.986	2.782			10.091	2.135					15.88483	2.018808
27														
28	11.300	2.146							10.787	4.045	16.806	5.792		
30														
32			24.493	3.962			25.809	4.219						
33	25.549	6.242			9.867	3.636			25.768	4.844			26.52324	6.091059
34														
35											27.045	5.792		
37					25.307	6.780	46.812	6.716						
38	48.199	4.214	51.775	4.558					49.141	7.600				
40											49.080	5.898	47.23174	5.899602
43					50.215	6.553								
44			73.890	4.350					76.881	6.863				
45							74.871	4.037						
46	75.505	7.430											78.343	7.898401
47											77.181	14.164		
49					75.915	6.902								
50														
51	101.526	5.911	100.491	7.600			98.497	4.151	100.129	5.274			98.85051	9.213441
52											99.910	6.435		
55					101.546	6.511								

Table 1. Corning Costar 96-well flat bottomed plate used to measure residual volume at various Z-axis manifold positions on an ELx405.

Plate: 96-well flat bottomed TC treated Corning Costar PN3603														
	DI Water		10 mM HEPES		100 mM HEPES		0.1% Tween 20		1% Tween 20		Delbecco's Media		Tyrode's Salt Solution	
Z Position	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL
23														
26	15.396	2.834	11.824	2.783	15.438	3.287	9.346	3.139			15.85018	2.882018	15.968	2.450505
27									9.727	3.019				
28														
30														
32														
33	27.535	7.253			25.175	4.979	24.687	4.957			24.50644	3.867981	25.3693	4.554627
34			27.306	5.138					26.149	6.715				
37														
38	50.392	6.070			48.770	6.215								
39			50.278	5.294			52.541	5.514	49.061	6.141	51.45591	5.809385	51.32769	5.445884
40														
43														
44	76.378	5.967			72.563	6.830								
45			75.865	8.396			76.402	7.148	75.952	7.134	75.45296	5.350846	75.66434	5.674358
46														
49														
50	98.251	9.477			99.194	7.117								
51			103.012	6.265			99.034	5.428	99.516	7.249	102.2118	6.688298	101.2623	5.981247
52														
55														

Table 2. Corning Costar 96-well flat bottomed plate Tissue Culture treated was used to measure residual volume at various Z-axis manifold positions on an ELx405.

Plate: 384-well flat bottomed Corning Costar PN3702										
	DI Water		10 mM HEPES		100 mM HEPES		0.1% Tween 20		1% Tween 20	
Z Position	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL	Residual Vol. (µL)	± µL
23										
26										
27										
28										
30										
32	4.910725	1.495	5.000	1.379	5.299	1.288				
33							5.500	3.305	5.211	1.764
34										
37	9.895	2.150	9.735698	1.813	9.860	1.722	9.947	3.158		
38									10.257	2.694
39										
40										
41							14.564	2.977		
42	15.111	2.373	14.94834	1.952	15.101	1.819			14.615	2.836
43										
44										
45										
46	19.655	2.562					20.320	2.425		
47			20.35504	2.306	20.584	2.119			20.353	3.085
50							24.934	3.170		
51	25.300	4.284944	24.996	2.658	25.152	2.434			25.033	3.304
52										
55										

Table 3. Corning Costar 384-well flat bottomed plate Tissue Culture treated was used to measure residual volume at various Z-axis manifold positions on an ELx405.

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