Cell washing performance of Thermo Scientific Wellwash Versa

Key Words:
- Cell wash
- Cell viability
- Adjustable dispensing
- Adjustable aspiration

This technical note reports a study of washing efficiency and cell viability after washing with a Thermo Scientific Wellwash Versa microplate washer.

Introduction

Wellwash Versa is a microplate washer for applications in research and routine testing laboratories. It provides reliable and secure washing of 96- or 384-well plates, for example ELISA, and washing of cells in 96-well plates.

For the demands of cell washing, such as in ion channel assays or cell-based ELISAs, it offers adjustable parameters, for example aspiration height, aspiration speed and drop wise dispensing. (Figure 1).

A double strip wash head is used to increase throughput.

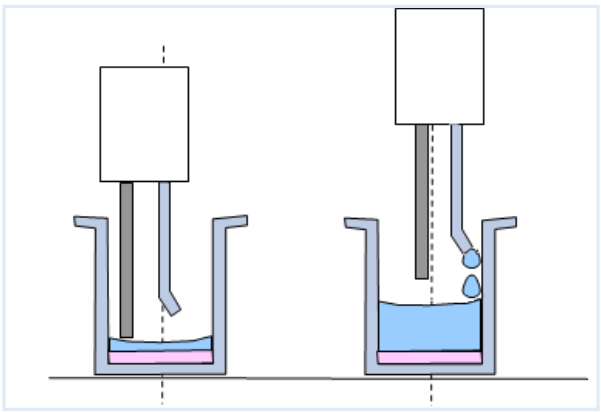

Figure 1. Aspiration mode, height, speed and time, and dispensing height and well position are adjustable in Wellwash Versa cell wash protocols.

This technical note describes a study of the washing efficiency of Wellwash Versa, and the effect of two parameters: aspiration speed and height, on the viability of human HeLa-S3 cells.

Washing efficiency of a microplate washer means how efficiently the washer is able to aspirate all the liquid in the well, i.e., leaving a low residual volume. Aspiration height defines the distance of the aspiration tip from the bottom of the well and aspiration speed the force used to aspirate the liquid from the well.

The parameter's aspiration height and speed must be optimally adjusted to avoid disrupting the delicate cell layer, but at the same time ensuring efficient washing.

The effect of washing was measured using a colorimetric method for determining the number of viable cells. In the assay the live cells reduce the MTS tetrazolium compound into a colored formazan product, which can then be detected by photometric measurement. The number of living cells per well were then compared to non-washed wells and the results were reported as a percentage of viable cells.

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Materials and methods

- HeLa-S3 human cervix carcinoma cells
- CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, code G3581)
- 96oF TC Nunclon D straight microplate with lid (Thermo Scientific, code 161093)
- F-12 Kaighn´s Modification-medium+ 10% FBS+ Penicillin/Streptomycin
- Wellwash Versa microplate washer, Thermo Scientific, code 5165010
- Multidrop Combi, reagent dispenser, Thermo Scientific, code 5840300
- Varioskan Flash multimode reader, Thermo Scientific, code 5250030

a) Washing efficiency

Washing efficiency was tested without cells by dispensing Ponceau S color solution and then washing the wells. Washing efficiency was calculated by comparing the volume of PonceauS left on the wells after washing to the initial dispensed volume of Ponceau S.

- 100 µl of 0.1% Ponceau S/PBS was dispensed into each well by Multidrop Combi. The plates were washed with the protocols described in Table 1.
- After washing, 200 µl of PBS was dispensed into each well and the absorbance was measured at 540 nm with Varioskan Flash.
- This result was compared to a previously made calibration curve of Ponceau S. From this residual volume, the washing efficiency was calculated by comparing it to the initial volume.

	Protocol			
Parameter	**Wash 1 x 600 µl 3 mm**	**Wash 2 x 600 µl 3 mm**	**Wash 1 x 600 µl 5 mm**	**Wash 2 x 600 µl 5 mm**
Wash head	2 x 8/96 (cell wash)	2 x 8/96 (cell wash)	2 x 8/96 (cell wash)	2 x 8/96 (cell wash)
Wash volume (µl)	600	600	600	600
Wash cycles	1	2	1	2
Wash mode	Plate	Plate	Plate	Plate
Aspirate height (mm)	6.1	6.1	8.1	8.1
Aspirate offset (mm)	-1.0	-1.0	-1.0	-1.0
Aspirate speed	Low	Low	Low	Low
Wash head speed (mm/s)	1	1	1	1
Aspirate time (s)	1	1	1	1
Dispense height start	7.1	7.1	9.1	9.1
Dispense height end	12.0	12.0	12.0	12.0
Dispense offset	1.0	1.0	1.0	1.0
Dispense tip touch	0	0	0	0
Final aspirate	Yes	Yes	Yes	Yes

Table 1. Parameters used in the test protocols

b) Effect of aspiration parameters

General

The HeLa-S3 cells were cultivated and the suspension (40,000 cells/ml) was dispensed into a Nunclon 96-well plate using a Multidrop Combi reagent dispenser. 100 µl of suspension was dispensed into all the test and control wells. The rest of the plate was left empty to function as blanks. The plates were incubated for 24 hours (+37°C/5% CO_2). The plates were washed with the media with Wellwash Versa (test wells). The control wells were left unwashed.

100 µl of media was added to all wells including the blanks. 20 µl of AQ reagent was added to all wells and the plate was incubated for 4 hours (+37°C/5% CO_2).

The absorbance values were measured using Varioskan Flash at the MTS's specific wavelength caused by excess cell debris, fingerprints and other nonspecific absorbance.

The results of the washed wells were compared to the control/non-washed wells (100% cell viability) and reported as viability percent.

1. Aspiration height

The effect of the aspiration height was tested by changing the aspiration height parameter from 2 to 5 mm in increments of 1 mm.

100 µl of the 40,000 cells/ml suspension was dispensed in 10 columns (two columns/each aspiration height). The remaining columns were used as blanks. The rest of the wash parameters were the same as in the washing 500 nm and at the reference wavelength 700 nm. The reference wavelength is used to subtract background efficiency test and detection as described in General above.

2. Aspiration speed

In this test, the effect of the aspiration speed for cell viability and washing efficiency was studied. Aspiration heights used were 2 and 3 mm from the bottom of the well and the aspiration speeds of low, medium and high. Two columns/aspiration speeds and the control were used. Altogether two plates/aspiration heights were tested.

The rest of the washing and detection parameters were as described above.

To demonstrate the possible effect of speed on loosely adherent cells, the test was repeated without letting the cells attach. After dispensing, the plate was centrifuged briefly to make the cells form a pellet to the bottom at the plate, and the test was repeated with identical parameters.

Results

a) Washing efficiency

The results of the washing efficiency test are reported in Figure 2.

The residual volumes, and thus the overall washing efficiency of Wellwash Versa, were very good with all protocols tested.

As expected, washing efficiency is improved when an additional washing cycle is added. However, washing efficiency is also reduced when the aspiration height is increased, i.e., the aspiration is performed at a longer distance from the well bottom.

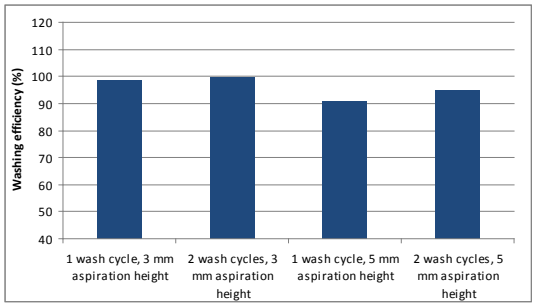

Figure 2. Washing efficiencies gained with the four protocols tested

b) Effect of aspiration parameters

1. Aspiration height

The results of the aspiration height test are reported in Figure 3.

As seen in the figure, the difference between the viability percentages between the different washing protocols is minimal. The difference can be ascribed to any natural variation in the cell assay, but a small correlation between the increased aspiration height and higher viability may be observed.

As expected, washing efficiency is slightly reduced when the aspiration height is increased.

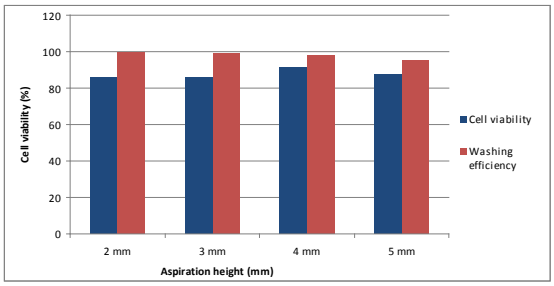

Figure 3. Aspiration height vs. cell viability with the tested protocols

2. Aspiration speed

The results of the aspiration speed test are reported in Figure 4.

The difference between the different speeds is negligible between the viability percents. This was to be expected, as the cell line is highly attachable.

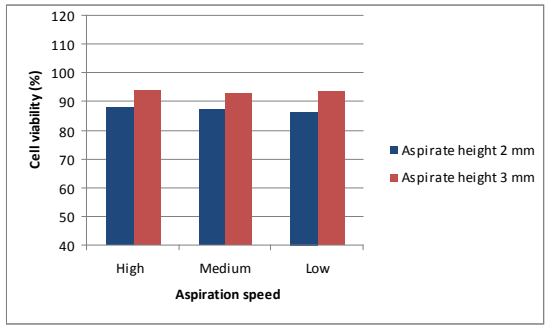

Figure 4. Aspiration speed vs. cell viability with adherent cells

With the "loosely adherent" cells, gentle aspiration clearly increases cell viability at an aspiration height of 2 mm. At an aspiration height of 3 mm the overall viability is clearly higher as expected, while the difference between the aspiration speeds is not clear anymore. The results of this test are shown in Figure 5.

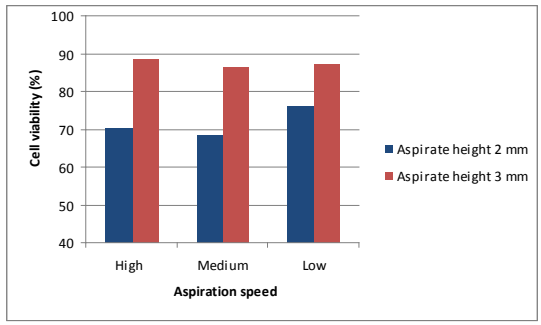

Figure 5. Aspiration speed vs. cell viability with the loosely adherent cells

Summary

- It is important that the wash is both gentle and efficient for optimal cell assay performance. The washer must offer the possibility of fine tuning the washing parameters to enable optimal washing conditions for different types of cell lines.
- Adjustable washing parameters together with the automation interface makes Wellwash Versa an efficient tool for screening a wide range of cell assay types.

References

CellTiter 96® AQueous One Solution Cell Proliferation Assay. Technical Bulletin, Promega corporation.

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