Technical Note: ANMICellwash

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 the cell viability after washing with Thermo Sientific Wellwash Versa microplate washer.

Introduction

Thermo Scientific Wellwash Versa is a microplate washer for applications in research and routine laboratories. It provides reliable and secure washing of 96- or 384-well plates for e.g. ELISA, and washing of cells on 96-well plates.
For the demands of cell washing like in ion channel assays or cell based ELISAs, it offers adjustable parameters for e.g. aspiration height, aspiration speed and drop wise dispensing. (Figure 1)
Double strip wash head is used to increase throughput.

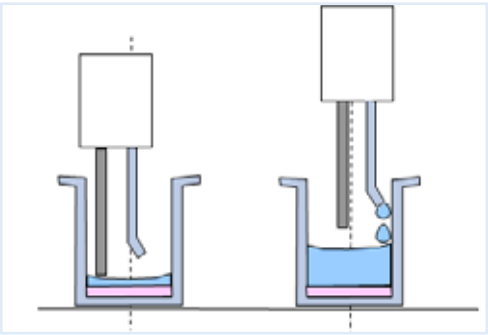

Figure 1. Aspiration mode, height, speed and time, and dispensing height and well position are adjustable in Wellwash Versa cell wash protocols.

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 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 parameters aspiration height and speed must be optimally adjusted to avoid disrupting the delicate cell layer, but at the same time to ensure efficient washing.
The affect of washing was measured by 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 a photometric measurement. The number of living cells/well were then compared to non-washed wells and the results are reported as a percentage of cells alive.

Materials and methods

- HeLa-S3 human cervix carcinoma cells
- CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, code G3581
- 96 F TC Nunclon D straight microplate w/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

The washing efficiency was tested without cells by dispensing Ponceau S color solution and washing the wells after that. Washing efficiency was calculated by comparing the volume of Ponceau S left on the wells after washing to the initial dispensed volume of Ponceau S.
- 100 µl of 0.1% Ponceau S/PBS were dispensed to each well by Multidrop Combi. The plates were washed with the protocols described on table 1.
- After washing, 200 µl of PBS was dispensed to 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.

Table 1. The parameters used in the test protocols

Protocol				
Parameter	**Wash 1x600µl 3mm**	**Wash 2x600µl 3mm**	**Wash 1x600 5 mm**	**Wash 2x600 5 mm**
Wash head	2x8/96 (cell wash)	2x8/96 (cell wash)	2x8/96 (cell wash)	2x8/96 (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

b) The effect of aspiration parameters

General

The HeLa-S3 cells were cultivated and the suspension (40000 cells/ml) was dispensed to Nunclon 96-well plate using Multidrop Combi reagent dispenser. 100 µl of suspension was dispensed to all test and control wells. The rest of the plate was left empty to function as blanks. The plates were incubated for 24 h (+37 oC/ 5% CO2). The plates were washed with the media with Wellwash Versa (test wells). The control wells were left unwahed.

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 h (+37oC/ 5% CO2).

The absorbance values were measured with Varioskan Flash at the MTSs specific wavelength 500 nm and at reference wavelength 700 nm. The reference wavelength is used to subtract background contributed 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 %.

1. Aspiration height

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

100 µl of the 40000 cells/ml suspension was dispensed to ten columns (2 columns/each aspiration height). The remaining columns were used as blanks. The rest of the wash parameters were as in washing efficiency test and detection as described on 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 Low, Medium and High. Two columns /aspiration speed and the control were used. Altogether two plates / aspiration height were made.

Rest of the washing and detection parameters were as described above.

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

Results

a)Washing efficiency

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

The residual volumes and therefore the overall washing efficiency of Wellwash Versa is was really good with all protocols tested.

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

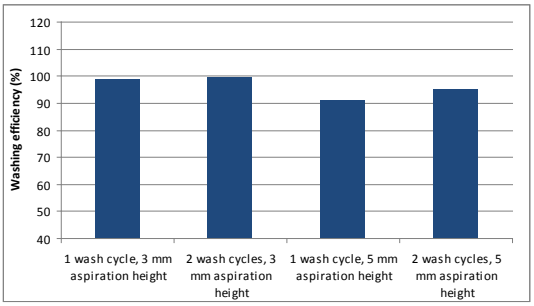

Figure 2. The washing efficiencies gained with the four protocols tested

b) The effect of aspiration parameters

1. Aspiration height

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

As can be seen from the figure, the difference between the viability percentages between the different washing protocols is quite small, Part of the difference is very likely due to the natural variation in the cell assay, but a small correlation

between the increased aspiration height and higher viability may be seen.
As expected, the washing efficiency is slightly decreased when the aspiration height is increased.

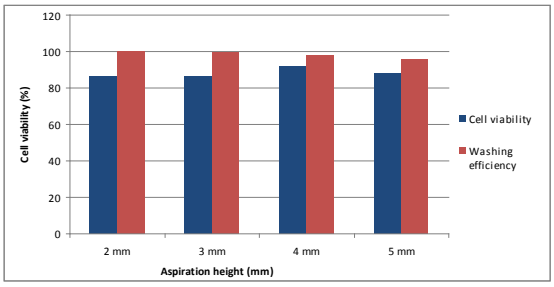

Figure 3. Aspiration height vs. cell viability with the tested protocols

2. Aspiration speed

The results of the aspiration speed test are reported on Figure 4.
The difference between the different speeds is not remarkable between the viability percents. This was to be expected, as the cell line is highly attachable.

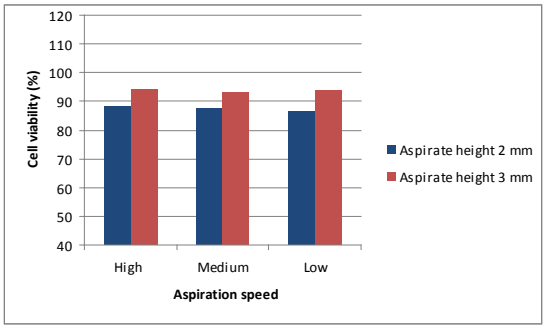

Figure 4. aspiration speed vs. cell viability with adherent cells

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

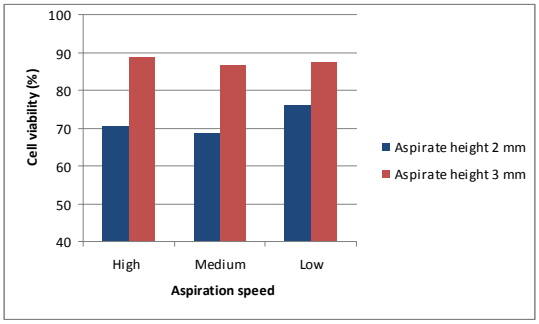

Figure 5. Aspiration speed vs. cell viability with the loosely adherent cells.

Summary

• Or optimal cell assays performance it is important that the wash is both gentle and efficient. This requires, that the washer offers the possibility to fine tune the washing parameters to enable the optimal washing conditions to different types of cell lines
• The adjustable washing parameters together with automation interface makes Wellwash Versa an efficient tool for screening type of cell assays.

References

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

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