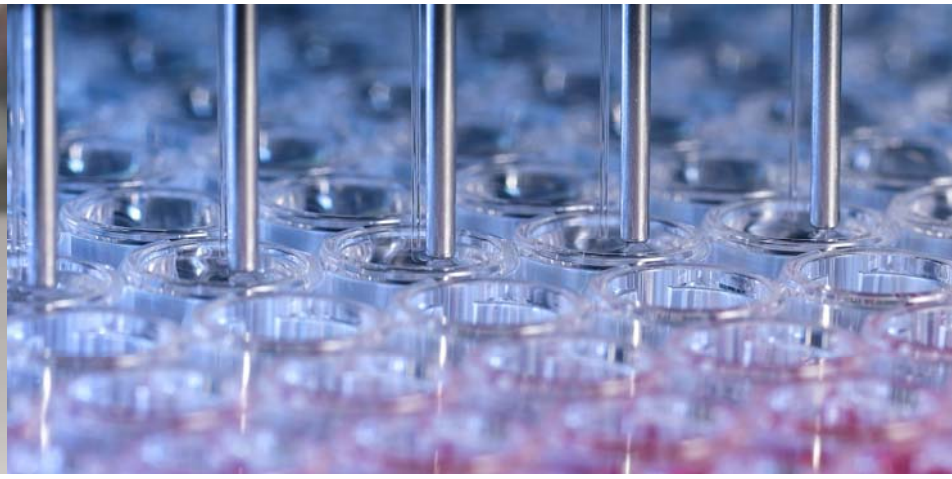
Gentle washing of cultured cells in microplates

Using the HydroFlex™ platform in drip mode



Introduction

This technical note describes how the Tecan HydroFlex platform was successfully evaluated for gentle washing of strongly adherent cell-line A431 as well as for the weakly adherent P815 cells in a 96-well format.

Tecan's new HydroFlex platform offers advanced features for a range of applications including vacuum filtration, magnetic bead separation and washing of microplates.

For gentle cell washing, the HydroFlex platform offers individual control of critical wash parameters such as speed settings for aspiration and dispense, as well as wash head positioning, allowing fine-tuning of the wash protocol to the specific cell-type used.

A very gentle drop-wise dispense mode combined with a move function optimizes the wash head position relative to the rising liquid level in the wells, thus minimizing cell detachment and ensuring good wash results, even with weakly adherent cell types.

For the cell-based assay described in this technical note, a HydroFlex platform, equipped with a standard wash head suitable for ELISA and cell washing, was used.

For cell-based assays, the HydroFlex platform allows to remove assay reactants with no or minor influence on the viability of the used cells.

To determine the wash efficiency and viability of the cells, experiments with strongly and semi-adherent cells were performed. Additionally the cell layers were monitored before as well as after washing to visually confirm the gentle wash procedure.

Material and Methods

Instrument

Tecan HydroFlex platform equipped with the standard 8-way manifold and two inlet channels for wash buffer

Microplates

Greiner Bio-One 96-well (Greiner Bio-One)

Reagents and Assay Performance

Reagents
Trypan Blue (TB), Dulbecco's Modified Eagle's Medium (DMEM, PAA Laboratories), 3-[4.5-dimethylthiazol-2-yl]-2.5-diphenyltetrazolium bromide (MTT)

Assay Protocol

Cell Culture
Human epidermoid carcinoma cells (A431, ATCC-No. CRL-1555) and mouse mastocytoma cells (P815, ATCC-No. TIB-64) were grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 mM HEPES, 4 mM L-glutamine, 1 mM Na-pyruvate, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 5 % (v/v) fetal calf serum (FCS) (all from PAA-Laboratories, Linz, Austria), in a humid atmosphere at 37° C and 7.5 % CO_2.

From the P815 cell line, adherent cells were selected by a series of washing procedures over a period of four weeks, where the supernatant (containing the suspension cells) was replaced by fresh DMEM supplemented as listed above.
For all assays, 2.10^4 cells of the respective cell line in 100 µl DMEM (supplemented as above) were seeded into each well of a 96-well microplate.

The wash efficiency of the HydroFlex Platform was determined by monitoring the removal of a coloured solution. Therefore, 20 µl of 0.05 % Trypan Blue (TB) was added to each well, the absorbance at 565 nm determined (= 100 %) and the washing procedure started. After washing, the remaining TB was determined again via absorbance measurements.

In order to determine cell viability, the MTT assay was performed on cells without a washing treatment (= 100 % viability) as well as on cells with a washing treatment (= x % viability). The MTT assay works on the principle that a metabolically active, viable cell reduces the soluble yellow tetrazolium salt MTT [3-(4.5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide] by the mitochondrial dehydrogenase activity, forming an insoluble dark formazan. The resulting absorbance values from the resolved formazan can be used to determine the viability and the number of viable cells.

Wash Programs
Each program listed below was tested for the effect of different wash volumes (600 µl in Program 1 and 800 µl Program 2) and the effect of repeating wash steps (one or two wash steps) on wash efficiency and on cell viability.

Program 1	
Wash 1x / 2x	
Z-position	10.5 mm + Move
Volume	**600 µl**
Head speed	1 mm / s
Wash rate	Drip mode
Aspirate	
Mode	Normal
Z-position	8500 µm
Time	1 s
Head speed	1 mm / s
Aspiration rate	1

Program 2	
Wash 1x / 2x	
Z-position	10.5 mm + Move
Volume	**800 µl**
Head speed	1 mm / s
Wash rate	Drip mode
Aspirate	
Mode	Normal
Z-position	8500 µm
Time	1 s
Head speed	1 mm / s
Aspiration rate	1

All runs were performed with A431 and P815 cells, respectively.

For all assays, $2 *10^4$ cells of the respective cell line in 100 µl supplemented DMEM were seeded into each well of a 96-well microplate.

Results

As an example figures 1 and 2 show light microscopic images of the weakly adherent cell line P815 & the adherent cell line A431 before and after performing Program 1 over above mentioned plates.

The images show that there are no holes within the cell layer.

These results confirm that the HydroFlex platform is well suitable for gentle processing of adherent and weakly adherent cell lines, ensuring intact cell layers after washing.

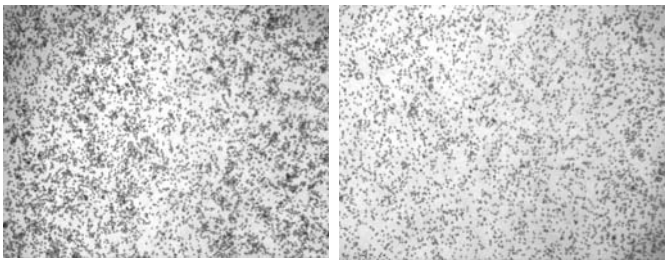


Figure 1: Weakly adherent cell line P815 before (left) and after washing (right) with PBS using Program 1

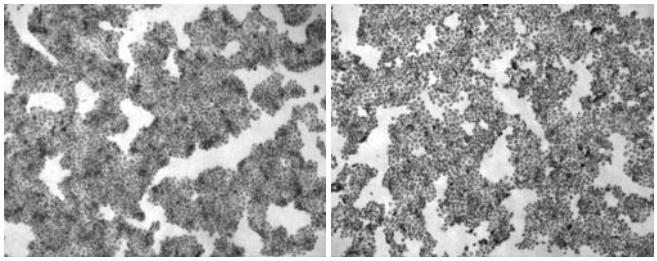


Figure 2: Adherent cell line A431 before (left) and after washing (right) with PBS using Program 1

Chart 1, below, shows the results of wash efficiency (TB-Test) and cell viability test (MTT test) after performing Program 1 or Program 2 with one or two washing steps. Each wash program was used to process two plates containing A431 and P815 cells respectively.

	Program 1		Program 2	
	Wash		Wash	
µl	**1x 600**	**2x 600**	**1x 800**	**2x 800**
A431 Cells				
Wash Efficiency [%]	95.6	98.3	97.3	98.6
Cell Viability [%]	81.0	84.1	76.1	75.96
P815 Cells				
Wash Efficiency [%]	94.9	99.1	97.4	99.0
Cell Viability [%]	73.0	70.7	73.8	64.7

Chart 1: Absorbance values [%] of TB and MTT after wash procedure

Photometric evaluation shows the alliance of high wash efficiency and a minor affect on the cells by using the HydroFlex platform.

Recovered cell rate lies above 75 % with A431 cells and 70 % with P815 cells (*Chart 1, above*).
The marginally lower recovery rates using Program 2 with 800µl volume to wash P815 cells are due to minor adhesion commonly seen with cell assays.

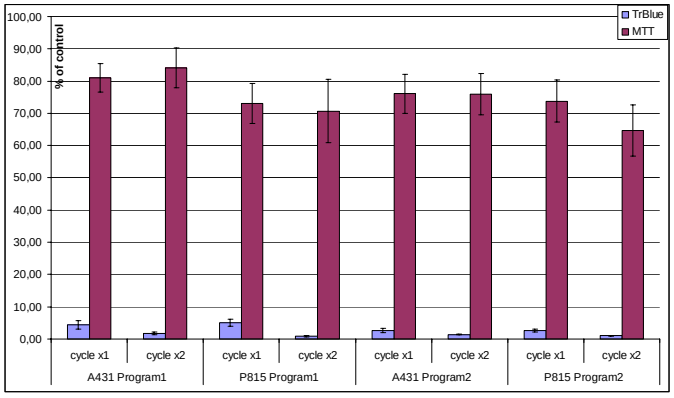


Figure 3: Content of TB and MTT after running Program 1 and Program 2 respectively

The data obtained show high washing efficiency of about 95 % and cell viability of more than 75 % using a one step wash and aspirate program.

Conclusion

The Tecan HydroFlex platform has demonstrated excellent performance for automated washing of adherent or weakly adherent cells, combining gentle and efficient washing with very low detachment rates and good cell viability.

Advanced control of critical wash parameters such as speed settings for aspiration and dispense, as well as wash head positioning, allows easy fine-tuning of wash conditions for a range of adherent, as well as weakly adherent cell-types cultured in microplates.

Acknowledgement

We would like to thank Mag. Tobias Kiesslich,
Mag. Juergen Berlanda (Research Group Prof. B. Krammer),
Department of Molecular Biology, University of Salzburg
www.uni-salzburg.at/pdt

March, 2007

Literature

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Caption

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395 065 V1.0, 05-2007