Technical Note

Performance Data demonstrating the Efficiency of the Tip Wash Station of the CyBi®-Well Family

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1. Abstract

The goal for this testing was to check the efficiency of the Tip Wash Station for the CyBi®-Well family. The settings of the pumping had to be optimized with respect to avoid the generation of air bubbles. Several systems were tested: water, buffer and DMSO.

The physical setting of the Tip Wash Station and the pipetting system was optimized in order to get a maximum performance without generating any air bubbles.

The performance of the Tip Wash Station was tested involving different liquids – which means the fluid in the originate wells of the compounds, the rinsing liquid used and the medium, which was in the destination wells after the washing. Water, 10%DMSO and a prototype biological buffer were investigated as media. The carry-over after the washing cycles was determined quantitatively.

The Tip-Wash-Station for the CyBi®-Well and CyBi®-Well vario showed a very good washing performance for a broad range of situations: more or less hydrophobic conditions and differences in detergent and protein content of source plate, washing liquid and destination plate.

A carry over of less than 0.006% can be expected when 2 washing cycles are used. For different types of liquids a single washing cycle can also be sufficient.

2. Introduction

For pipetting instruments, which offer the possibility to re-use tips, a washing station and its proper performance is crucial.
This performance is determined by different factors: the settings of instrumental parameters (like speeds, immersion depths), the process itself (how many steps?) and the types of liquids involved (e.g. water, organic solvents or detergents).
Some of these parameters can be optimized, which is shown in this application note.

The experiments in principle were performed as follows: Using the CyBi®-Well, a dye solution in a microplate (the "dye plate") was aspirated and dispensed back into this plate. Then a washing procedure was performed applying different amounts of cycles. Afterwards an aspirate-dispense cycle was performed in a microplate consisting of wells filled with different liquids (water and DMSO) (the "buffer plate").

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3. Materials:

Tip-Wash-Station:	Tip-Wash-Station 96 from CyBio
Plates:	Greiner 96 well plates, polystyrene, black, medium binding #655076
Tips:	CyB®-Tips 96 , 250 µL SW (46 mm)
Sealing sheets:	Nunc „"Fasson S695", #236269
Dye:	Flourescein-Sodium, Fluka, #46960
DMSO:	Dimethlysulfoxide "SeccoSolv, Merck, #1.02931.100
Pluronic:	PluronicF68, Sigma #P2443
BSA:	Albumin Bovine, Sigma, #A3509
Reader:	BMG Polarstar
Zentrifuge:	Sigma GK15, 1000 rpm, 20°C

The buffer consisted of 0.1% BSA, 150 mM NaCl and 0.01% Pluronic.

4. Abbrevations and Formulae:

STD	standard deviation
MW	mean
CV	coefficient of variance, STD / MW * 100%
rpm	rounds per minute

5. Methods

EXPERIMENTAL PROCEDURE:

Flourescein was diluted (100 µM) in three different liquids: Water; DMSO and buffer. The first 6 rows of a 96 well microplate ("dye plate") were filled row-wise according to the following scheme:

W	W	W	W	W	W	W	W	W	W	W	W
W	W	W	W	W	W	W	W	W	W	W	W
D	D	D	D	D	D	D	D	D	D	D	D
D	D	D	D	D	D	D	D	D	D	D	D
B	B	B	B	B	B	B	B	B	B	B	B
B	B	B	B	B	B	B	B	B	B	B	B

Fig1: "dye plate" filled with Water (w), DMSO (d) and buffer (b). Each well contained 100 µM fluorescein. Volume: 150 µL

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A second set of microplates ("buffer plate") as destination plates were prepared by filling 3 different liquids (Water, DMSO and buffer) column-wise into the wells (see Fig.2).

W	W	W	W	D	D	D	D	B	B	B	B
W	W	W	W	D	D	D	D	B	B	B	B
W	W	W	W	D	D	D	D	B	B	B	B
W	W	W	W	D	D	D	D	B	B	B	B
W	W	W	W	D	D	D	D	B	B	B	B
W	W	W	W	D	D	D	D	B	B	B	B

Fig2: "buffer plate" filled with Water (w), DMSO (d) and buffer (b). Volume: 150 µL

Combining these two plate layouts, a series of results involving different origins of the contaminant (fluorescein) and different destinations could be generated in a single run.

The experimental process consisted of four steps:

a) Insert new tips into the CyBi®-Well and aspirate 100µL from the "dye plate" then dispense this volume back into the "dye plate".

b) Run 1, 2,or 3 "Rinse/Mix" cycles at the Tip Wash Station using the appropriate washing fluid (DMSO or water).
Settings for the pipette tips: volume 100 µL, aspiration speed 50 rpm during "Rinse/Mix" procedure, dispensing speed 100 rpm – all other parameters were used at the default values.
Setting for the pumps transporting the rinsing fluid the following parameters were found: speed of the inlet-pump at 50 rpm and speed at the outlet at 200 rpm.

c) Use a freshly prepared "buffer plate" and aspirate 100µL from the "buffer plate" then dispense this volume back into the "buffer plate".

d) Perform a measurement of the "buffer plate" at the reader (fluorescence 488/520 nm, 10 flashes, gain 55).

Concentration determination:
A calibration curve of flourescein in water was generated using a concentration range from 1 to 30 nM. The measurement conditions (micro plate, reader parameters) were equivalent to those used by the washing experiments.

Readout calculation "Remaining Fluorescence":
For every "buffer plate" measured there was the well-wise difference of the fluorescence values to the mean of a series of fresh (non-contaminated) "buffer plates" calculated in order to keep the different experiments comparable. This value is referred to as "**remaining fluorescence**" throughout the text.

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6. Results

Settings of the pumping system:
The following settings were found to be optimal for the washing steps.
For the tips: Aspiration speed 50 rpm during the "Rinse/Mix" procedure, dispensing speed 100 rpm – all other parameters were used as its default values. The aspiration volume was 100 µL.
For the pumps transporting the rinsing fluid the following parameters were found: speed of inlet pump at 50 rpm and speed outlet at 200 rpm.

Calibration curve
Fig3. shows the calibration curve, which is linear from approximately 3 nM upwards.

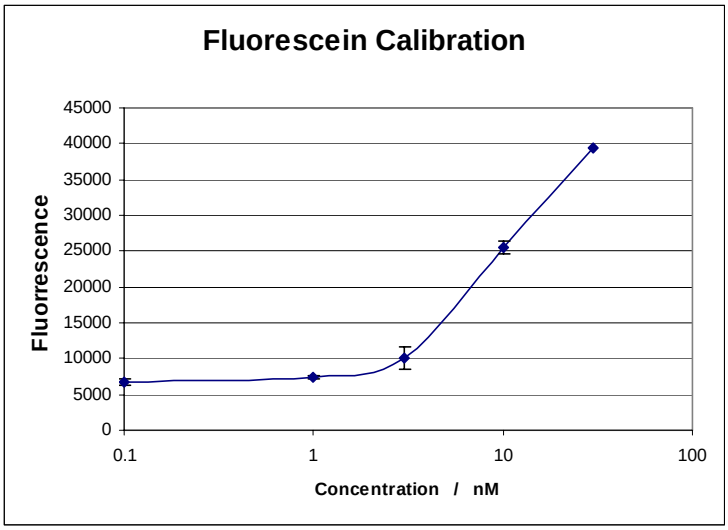

Fig3: Calibration curve of fluorescein in water

For an estimation of the fluorescein-carryover the readouts were converted into concentrations. The detection limit of this system was estimated at 3 nM fluorescein (which means readouts of 10000). Thus, a minimum detectable carry-over with this system is at 0.003% - based on a fluorescein concentration of 100 µM in the "dye plate".

Washout parameters

The detailed physical parameters for the pumps etc. are given in the methods. These balance the filling speed of the Tip Wash Station with the aspiration speed of the pipette tips in a way that no air bubbles are generated, which would have an impact on the washing effectiveness.
The intention was, not to alter too much of the default settings in order to make the user's life as simple as possible. Therefore only the speeds for the rinsing pumps and of the piston tips during aspiration and dispensing were altered.

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Washout performance

The Tip Wash Station showed an excellent performance. In general, when 2 washing cycles ("Rinse/Mix" cycles) were used, the Tip Wash Station works fine for all different scenarios – the remaining concentration in the destination wells were less than 0.003% of the original dye solution.

A typical result is shown in Fig.4.

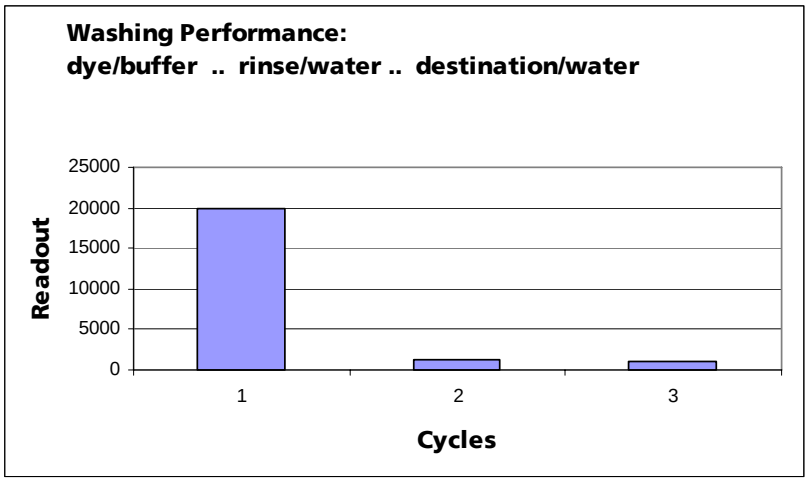

Fig4: Remaining fluorescence (see methods for calculation) after 1,2 and 3 washing cycles.

In this experiment the dye was diluted in buffer, the rinsing fluid was water and the fluid in the "buffer plate" was water. As can be seen after two washing cycles, there was virtually no remaining fluorescence observable. The concentration after applying one cycle is about 6 nM, which is 0.006% carry-over.

Difference between different solvent systems

Several measurements were performed while varying the different media (DMSO, water and buffer). For each of the experimental settings 1, 2 and 3 washing cycles were investigated.

The following list gives an overview of the experiments conducted:

Dye dissolved in	Rinsing fluid	Destination medium
Water	Water	Water
DMSO	Water	Water
Buffer	Water	Water
Water	DMSO	Water
DMSO	DMSO	Water
Buffer	DMSO	Water
Water	Water	DMSO
DMSO	Water	DMSO
Buffer	Water	DMSO
Water	DMSO	DMSO
DMSO	DMSO	DMSO
Buffer	DMSO	DMSO

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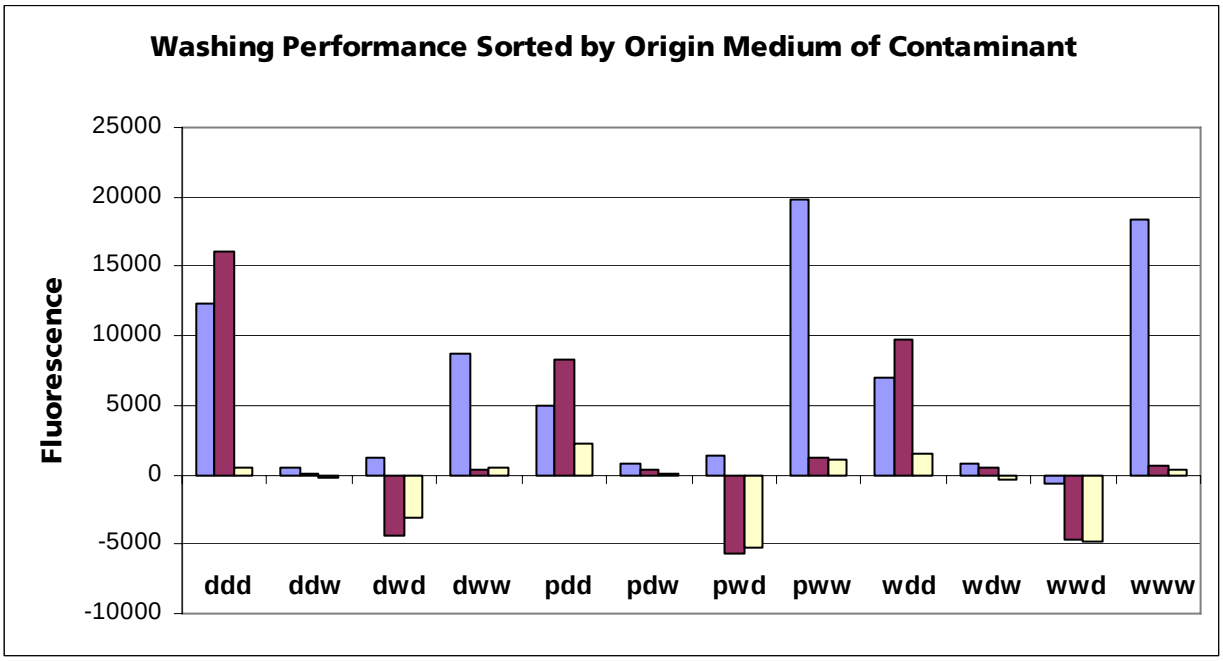
Looking at the readouts of all results **sorted by the original medium** used (Fig.5) there are 3 groups (A,B,C) visible each showing a similar pattern. Each group consists of 4 triples of washing cycles. Group **A** stands for DMSO as origin fluid, Group **B** for buffer and group **C** for water. In each group the sorting of the experiments is the same so that similar patterns arise when there is a similarity in the washing performance for the different source media.
Thus, it can be concluded that the origin of the contaminant does not matter – whether it is water, DMSO or buffer since the "washout-pattern" is always the same for all of these media.



(origin fluid) : **A** (DMSO) **B**(buffer) **C**(water)

Fig5: Results of the experiments sorted by the origin medium of the contaminant. The triple groups of columns show 1x washing, 2x washing and 3x washing cycles. Key to the abbreviations like "ddd" etc.: The letters indicate the type of medium (p-buffer, d-DMSO, w-water). The first letter stands for the originate medium used (in the "dye plate"), the second letter means the rinsing fluid and the third letter stands for the destination medium in the "buffer plate"). For example "pwd" means an experiment with buffer as originate medium (fluorescein diluted in buffer in the "dye plate"), with water as rinsing fluid and DMSO as recipient in the "buffer plate".

All the remaining fluorescence values are below 20000 – even those after a single washing. This means that the concentrations found in the "buffer plates" after the rinsing is less than 0.006% of the concentration in the origin "dye plates" since the origin concentration was 100 µM and the remaining fluorescence of 20000 correspond to approximate concentration of 6 nM.
Thus, an excellent performance of the Tip Wash Station could be demonstrated.

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Having **sorted** the results according to the **destination fluid** (Fig.6) there are 4 groups visible, each consisting of 9 experiments (3 experimental setups, each consisting the testing of 1,2 and 3 washing cycles). For group A and B there was DMSO as recipient medium, for the groups C and D it was water. For the groups A and C the **rinsing liquid** was DMSO and for the groups B and D water was used for rinsing.

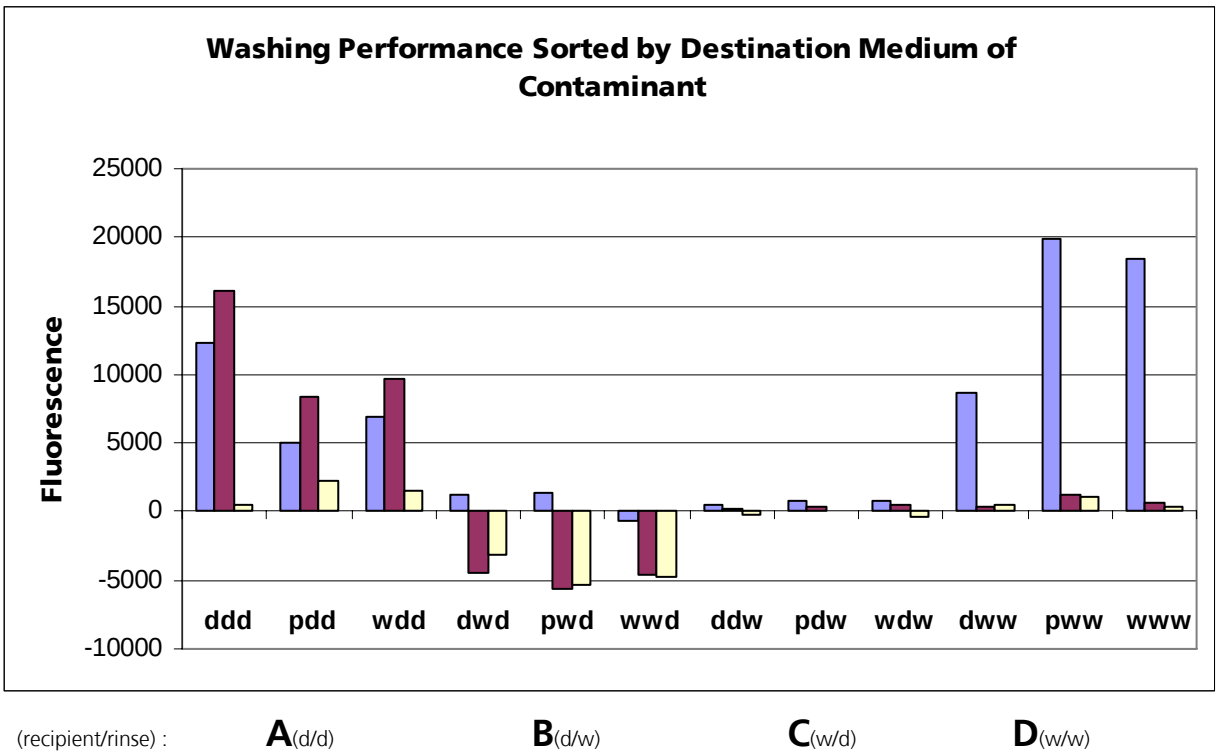

Fig6: Results of the experiments sorted by the destination of the contaminant (the contents of the "buffer plate"). The triple groups of columns show 1x washing, 2x washing and 3x washing cycles. Key to the abbreviations like "ddd" etc.: The letters indicate the type of medium (p-buffer, d-DMSO, w-water). The first letter stands for the originate medium used (in the "dye plate"), the second letter means the rinsing fluid and the third letter stands for the destination medium in the "buffer plate").. For example "pwd" means an experiment with buffer as originate medium (fluorescein diluted in buffer in the "dye plate"), with water as rinsing fluid and DMSO as recipient in the "buffer plate".

If the rinsing fluid and recipient liquid are the same, the situation is as follows. If washing with water and if the recipient liquid is water as well (group D) then at least 2 cycles are necessary for resulting in a signal at or below the detection limit. The same holds for a DMSO recipient and DMSO as rinsing fluid (group A). If the rinsing fluid is of a different type as the recipient liquid then a single cycle is sufficient (groups B and C).

Some statements can be concluded.
The origin medium of the dye (DMSO, water or buffer) did not show a significant impact on the performance. The best results were seen, when 10% DMSO was the rinsing medium and water was in the destination wells. Good results are found when the rinsing fluid is different from the recipient fluid.

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