APPLICATION NOTE

Start-up Routine and Tip Washing Efficiency Data of the Active Deep Well Tip Wash Station 384

Katrin Undisz PhD, CyBio AG

Key words

CyBi®-Well and CyBi®-Well vario, tip washing efficiency, different washing liquids, optimized start-up and method settings, washing liquid consumption, fluorescence, buffer, DMSO

Summary

In this Application Note the tip washing efficiency of the active deep well Tip Wash Station 384 was determined quantitatively with optimized pump settings and different washing liquids after different numbers of washing cycles using a fluorescence read-out. Furthermore the start-up routine for the active deep well Tip Wash Station 384 was described in detail. The carry-over after one washing cycle was less than 0.006 % for all of the 8 tested washing liquids. Compared to pure water the addition of detergents as well as the use of pure DMSO did not much influenced the carry-over, while the addition of 10% Ethanol as well as 10% DMSO improved it slightly. Rinsing with pure ethanol resulted in the highest carry-over in our test.

After 3 washing cycles with water the carry-over for Fluorescein stock solutions both in buffer and in DMSO was less than 0,001 %, thus touching the detection limit of the fluorescence method that was used in our experiments. These data agree with the test results of the active shallow well Tip Wash Station 96 (1) and demonstrate the excellent washing efficiency of the active deep well Tip Wash Station 384 (see Fig.1).

Introduction

The proper function of a tip wash station corresponding to the tip number and tip length is a condition for the multiple use of tips without risk of cross contamination. The possibility of automated and effective washing of disposable tips seriously reduces the costs for many liquid handling applications in drug research and life science. The efficiency of the tip washing procedure directly influences the success of the experiments.

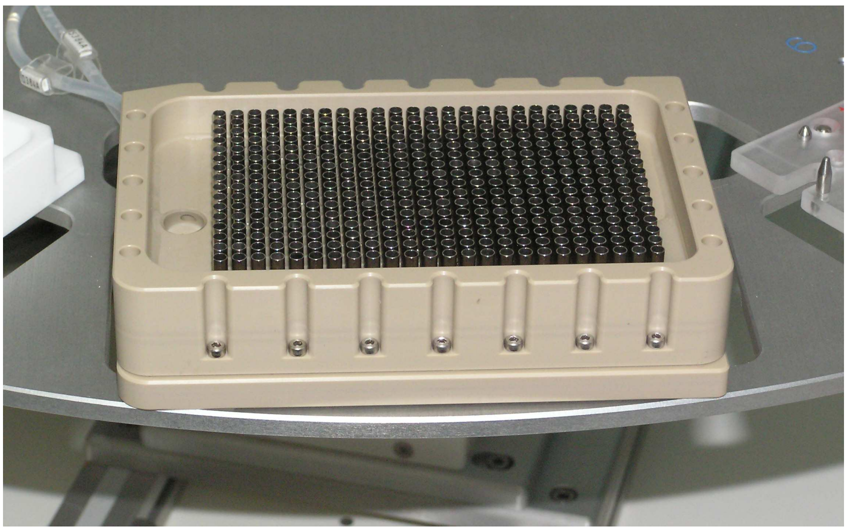

Fig.1: Tip wash trough of the active deep well Tip Wash Station 384

APPLICATION NOTE

As a sensitive application example in molecular biology siRNA transfection was selected to demonstrate the high efficiency of CyBio´s active Tip Wash Station. It could be demonstrated, that one washing cycle with the active shallow well Tip Wash Station 96 was sufficient for the removal of any remnants of a siRNA transfection complex thus allowing tip washing as a suitable alternative to tip changing in siRNA transfection experiments (2).

Materials and Reagents

- CyBi®-Well vario with 384/25 µL head and active deep well Tip Wash Station 384
- 25 µL tips (CyBio # OL 2001-25-250)
- 384 well plates PS black (Greiner bio-one # 781 076)
- Fluorescein-Sodium (Standard Fluka # 46960)
- Fluorescein, free acid (Standard Fluka # 46955)
- PBS (Sigma # P3813)
- DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)
- Ethanol (> 99.8 %, Roth, # 5054.1)
- RBS 35 Konzentrat (Roth, # 9238.2)
- Triton® X-100 (Merck Darmstadt, # 1.12298.0101)
- 8 channel reagent reservoir, high profile (Nerbe plus # 04.072.0380)
- PolarStar (BMG Labtechnologies) with filter set 485 nm (ex) and 520 nm (em)

Methods

Fluorescence measurement

All fluorescence measurements were performed in black 384 well plates (final volume 40 µL per well). To obtain a test solution with low surface tension and higher viscosity 1 mM Fluorescein was dissolved in DMSO, for a test solution with high surface tension and lower viscosity 1 mM Fluorescein-Sodium was dissolved in PBS buffer. Both test solutions and the corresponding blanks were arranged in a test plate as shown in Fig.2.

<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th><th>13</th><th>14</th><th>15</th><th>16</th><th>17</th><th>18</th><th>19</th><th>20</th><th>21</th><th>22</th><th>23</th><th>24</th></tr>
<tr><td>A</td><td colspan="2" rowspan="16">PBS</td><td colspan="8" rowspan="16">1 mM Fluorescein-Sodium test solution in PBS</td><td colspan="2" rowspan="16">PBS</td><td colspan="2" rowspan="16">DMSO</td><td colspan="8" rowspan="16">1 mM Fluorescein test solution in DMSO</td><td colspan="2" rowspan="16">DMSO</td></tr>
<tr><td>B</td></tr>
<tr><td>C</td></tr>
<tr><td>D</td></tr>
<tr><td>E</td></tr>
<tr><td>F</td></tr>
<tr><td>G</td></tr>
<tr><td>H</td></tr>
<tr><td>I</td></tr>
<tr><td>J</td></tr>
<tr><td>K</td></tr>
<tr><td>L</td></tr>
<tr><td>M</td></tr>
<tr><td>N</td></tr>
<tr><td>O</td></tr>
<tr><td>P</td></tr>
</table>

Fig.2: 384 well test plate layout, 40 µL liquid per well

APPLICATION NOTE

It is known that the remaining carry-over in the tips already without tip washing is less than 1 % in every case. Thus from both 1 mM Fluorescein test solutions two calibration curves were generated starting from 10 µM Fluorescein (corresponding to 1% carry-over, reader gain = 25) down to 1 nM Fluorescein (corresponding to 0.0001 % carry-over, reader gain = 45) thus allowing a quantification of the carry-over of both 1 mM Fluorescein test solutions finally over six orders of magnitude down to 1 nM Fluorescein (see Fig.3 and Fig.4).

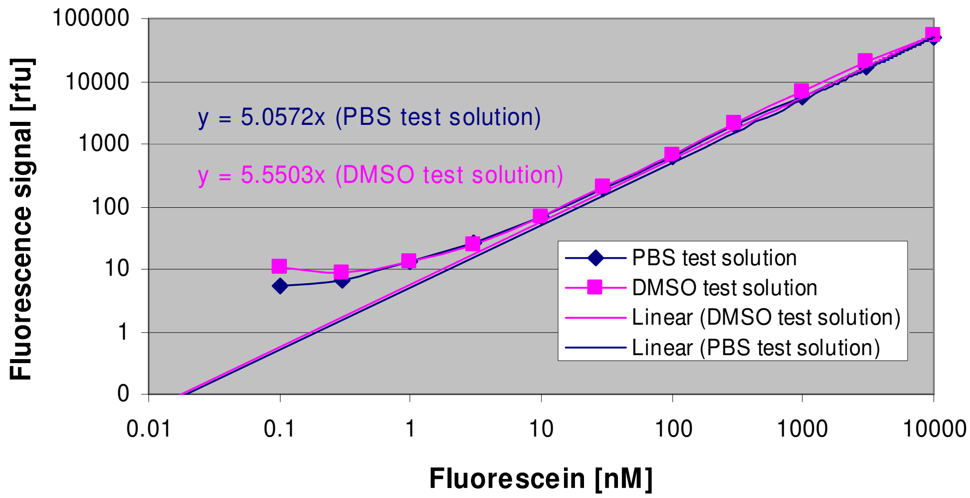

Fig.3: Fluorescein calibration curves from test solutions in PBS and DMSO, (BMG PolarStar, raw data - blank, gain 25, n=16), the linear range at gain setting 25 is 10 µM - 10 nM.

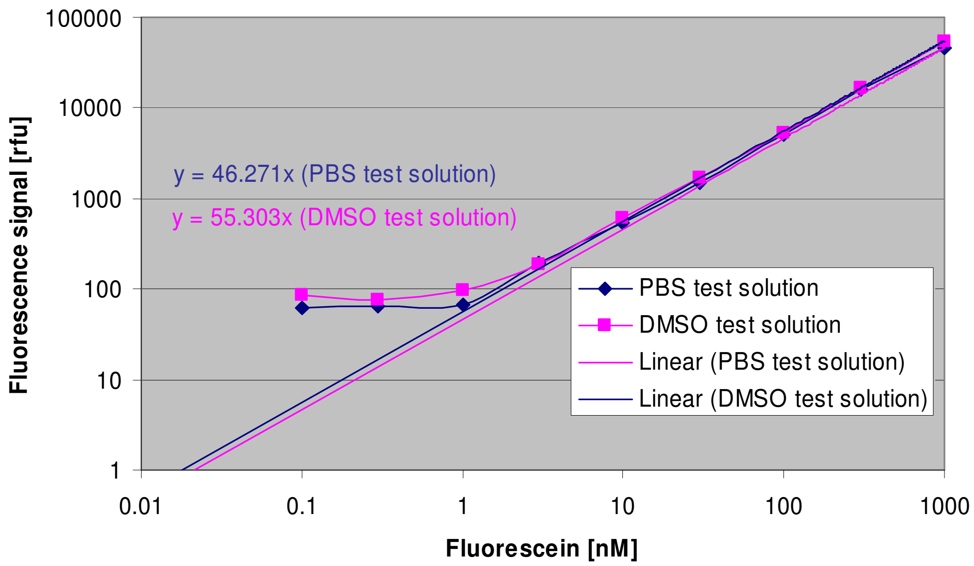

Fig.4: Fluorescein calibration curves from test solutions in PBS and DMSO, (BMG PolarStar, raw data - blank, gain 45, n=16), the linear range at gain setting 45 is 1 µM - 1 nM.

APPLICATION NOTE

Comparison of the wash efficiency of different washing liquids

To compare the efficiency of 8 different washing liquids a deep well 8 channel wash liquid reservoir was prepared as described in Fig.5.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1 = deionized water																							
B																								
C	2 = deionized water + 0.1 % RBS 35																							
D																								
E	3 = deionized water + 1 % RBS 35																							
F																								
G	4 = deionized water + 0.01 % Triton X100																							
H																								
I	5 = deionized water + 10 % Ethanol																							
J																								
K	6 = deionized water + 10 % DMSO																							
L																								
M	7 = 100 % Ethanol																							
N																								
O	8 = 100 % DMSO																							
P																								

Fig.5: Wash reservoir layout, 8 channel reagent reservoir with 25 mL washing liquid per row.

Using the CyBi®-Well vario with 384/25 µL head and 25 µL tips at first 20 µL were aspirated out of the test plate (Fig.2) and dispensed back with overstroke and tip touch into the test plate. With the same set of tips in a second step 25 µL were aspirated out of the wash reservoir (Fig.5) and dispensed back with overstroke and tip touch into the wash reservoir. In a third step 25 µL were resuspended 3 times in a measure plate that was prefilled with 40 µL PBS per well. The remaining fluorescence was measured and after blank subtraction the carry over was calculated using the corresponding calibration curves. The protocol is described schematically in Fig.6.

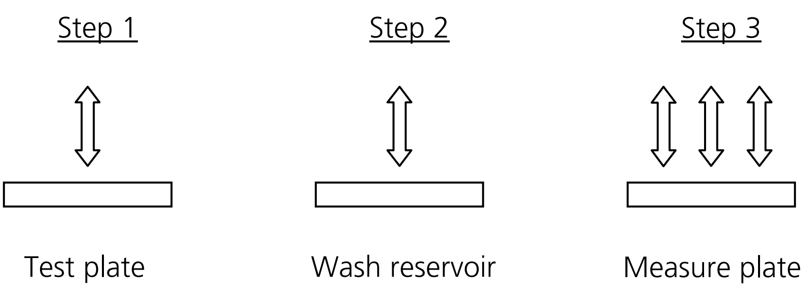

Fig.6: Protocol scheme to compare the wash efficiency of different washing liquids.

APPLICATION NOTE

Start-up routine and pump settings of the active deep well Tip Wash Station

All washing liquids have to be particle and fluff free. Before every use the active deep well Tip Wash Station 384 has to be primed carefully to avoid the blocking of the chimneys by tight air bubbles and to secure constant flow of the washing liquid. For these purposes it is recommended to perform three washing cycles with a final source pump speed of at least 200 rpm with the tips immersed as deep as possible in the chimneys. After that the correct flow of the wash liquid in all chimneys has to be checked visually. If single chimneys are still blocked by air bubbles these should be evacuated through the adequate chimney using a single channel manual 1 mL pipette.

If the Tip Wash Station has been primed correctly the wash liquid flows constantly. Please refill the wash liquid vessel early enough to avoid the penetration of air into the tube system.

For the normal wash routine the final source pump speed should be at least 150 rpm. To avoid an overflow of the trough the final speed of the drain pump should be at least 50 rpm higher than that of the source pump. In Tab.1 average values for the time and the amount of wash liquid for different wash cycles and different source pump speeds at piston speed 300 rpm and stage speed 140 rpm are summarized.

	1 Washing cycle	2 Washing cycles	3 Washing cycles
Time [s]	14	28	42
Washing liquid [mL] at final source pump speed 150 rpm	40.4	79.5	119.5
Washing liquid [mL] at final source pump speed 200 rpm	52.5	105.0	157.5
Washing liquid [mL] at final source pump speed 250 rpm	66.6	132.1	199.1

Tab.1: Average values for the time and the amount of washing liquid for different washing cycles and different source pump speeds at piston speed 300 rpm and stage speed 140 rpm, n=3, final drain pump speed setting always was 50 rpm higher than final source pump speed.

Determination of the carry-over after different washing cycles

Using the CyBi®-Well vario with 384/25 µL head and 25 µL tips at first 20 µL were aspirated out of the test plate (Fig.2) and dispensed back with overstroke and tip touch into the test plate. Then with the same set of tips 25 µL were resuspended 3 times in a measure plate that was prefilled with 40 µL PBS per well to determine the remaining fluorescence without washing. With a new set of tips the aspiration and dispense procedure out of the test plate was repeated and one washing cycle in the active deep well Tip Wash Station was performed with deeply immersed tips and a final source pump speed of 200. Then the tips were resuspended again 3 times in a prefilled measure plate and the carry over after 1 washing cycle was determined. The same procedure was repeated each time with a new set of tips with 2 and 3 washing cycles. After blank subtraction the carry over was calculated using the corresponding calibration curves. The protocol to determine the carry-over after different washing cycles is described schematically in Fig.7.

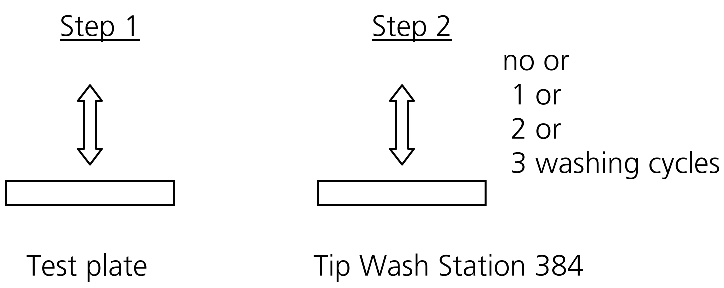

Fig.7: Protocol scheme to determine the carry-over after different washing cycles.

Results and Discussion

The carry-over of 8 different washing liquids after one washing cycle is shown in detail in Fig.8 and Tab.2. It was less than 0.006 % for all of the 8 tested washing liquids. At our experimental conditions the addition of detergents as well as the use of pure DMSO did not much influence the carry-over compared to pure water, while the addition of 10 % DMSO reduced the carry-over slightly for the PBS test solution. Rinsing with pure ethanol resulted in the highest carry-over in our test for both test solutions.

These results were obtained with Fluorescein as test compound dissolved in PBS and DMSO, respectively. The carry-over may differ with other compound solutions depending on their specific properties and has to be checked individually. The addition of detergents is recommended if sticky proteins have to be handled. The addition of solvents may improve the cleaning effect for highly hydrophobic compound solutions.

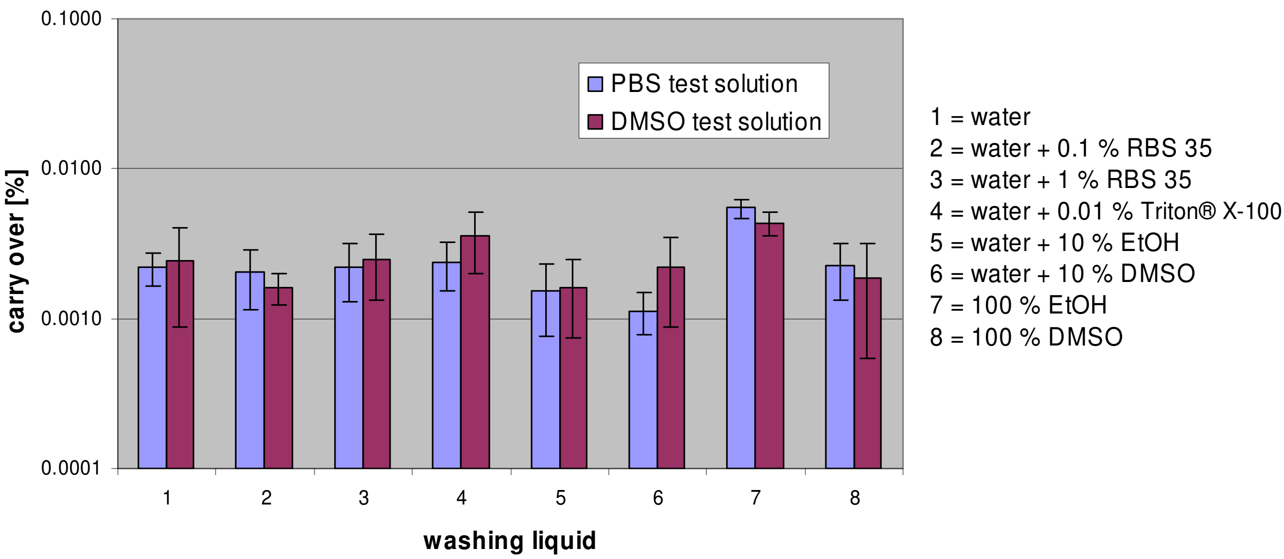

Fig.8: Graph of the carry-over of 8 different washing liquids after one washing cycle (n=16), data see also Tab.2.

	Washing liquid	Fluorescein test solution in PBS carry-over after 1x washing [%]	Fluorescein test solution in DMSO carry-over after 1x washing [%]
1	Deionized water	0.00219	0.00245
2	Water + 0.1% RBS 35	0.00203	0.00162
3	Water + 1 % RBS 35	0.00221	0.00251
4	Water + 0.01 % Triton® X-100	0.00237	0.00354
5	Water + 10 % EtOH	0.00154	0.00161
6	Water + 10 % DMSO	0.00113	0.00220
7	100 % EtOH	0.00545	0.00433
8	100 % DMSO	0.00225	0.00184

Tab.2: Carry-over of 8 different washing liquids after one washing cycle (n=16), graph see also Fig.8.

The carry-over after different washing cycles was determined with deionized water as washing liquid. The results are shown in Fig.9 and Tab3.

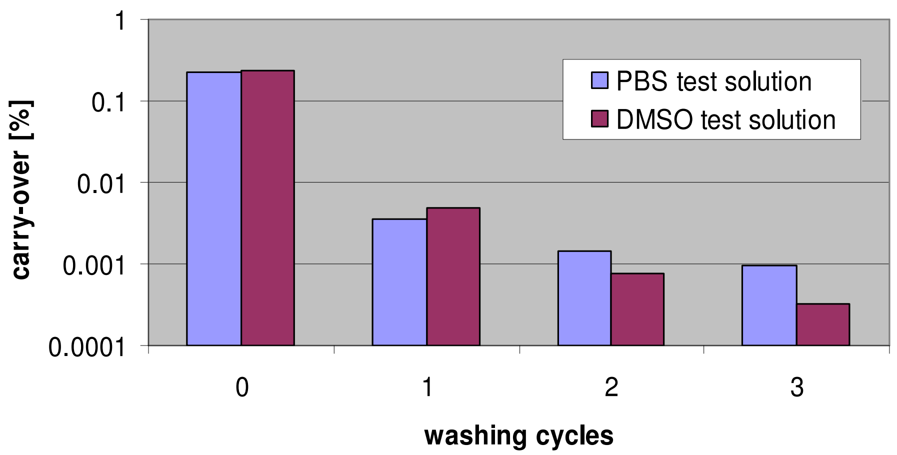

Fig.9: Graph of the carry-over of Fluorescein test solution solved in PBS and DMSO, respectively, after different washing cycles with deionized water (n=128), data see also Tab.3.

N° of washing cycles	Fluorescein test solution in PBS carry-over [%]	Fluorescein test solution in PBS CV [%], n= 128	Fluorescein test solution in DMSO carry-over [%]	Fluorescein test solution in DMSO CV [%], n= 128
0	0.2082	15	0.2045	48
1	0.00354	15	0.0048	32
2	0.00145	13	0.00076	27
3	0.00095	13	0.00033	32

Tab.3: Carry-over of Fluorescein test solution solved in PBS and DMSO, respectively, after different washing cycles with deionized water (n=128), graph see also Fig.9

APPLICATION NOTE

At our experimental conditions the carry-over without tip washing was 0.2082 % for the PBS test solution and 0.2045 % for the DMSO test solution. That means that at average about 80 nL liquid remain at the 25 µL tips after aspiration of 20 µL and dispensing with overstroke and tip touch. The mean carry-over for the PBS and the DMSO test solution was very similar, but the variation from well to well was three times higher for the DMSO test solution.

For both test solutions the first washing cycle reduced the remaining carry-over for nearly two orders of magnitude to less than 0.005 %. After two washing cycles the carry-over was less than 0.002 %. The third washing cycle further reduced it to less than 0.001 %.

These data agree with the test results of the active shallow well Tip Wash Station 96 (1) and demonstrate the excellent washing efficiency of the active deep well Tip Wash Station 384.

References:

1. Prüfer, H. and Busch, M. (2007); "Performance Data demonstrating the Efficiency of the Tip Wash Station of the CyBi®-Well Family", TechNote CyBio AG, www.cybio-ag.com

2. Hewitson, M., Hermann, H. and Benn, N. "How to perform High Throughput siRNA Transfections", common poster of Cenix Bioscience and CyBio AG at the RNAi Europe 20.-21.September 2007 Barcelona, Spain, www.cybio-ag.com