TECHNICAL NOTE

# Highly Efficient Washing of Microwell Plates Using Centrifugal Forces

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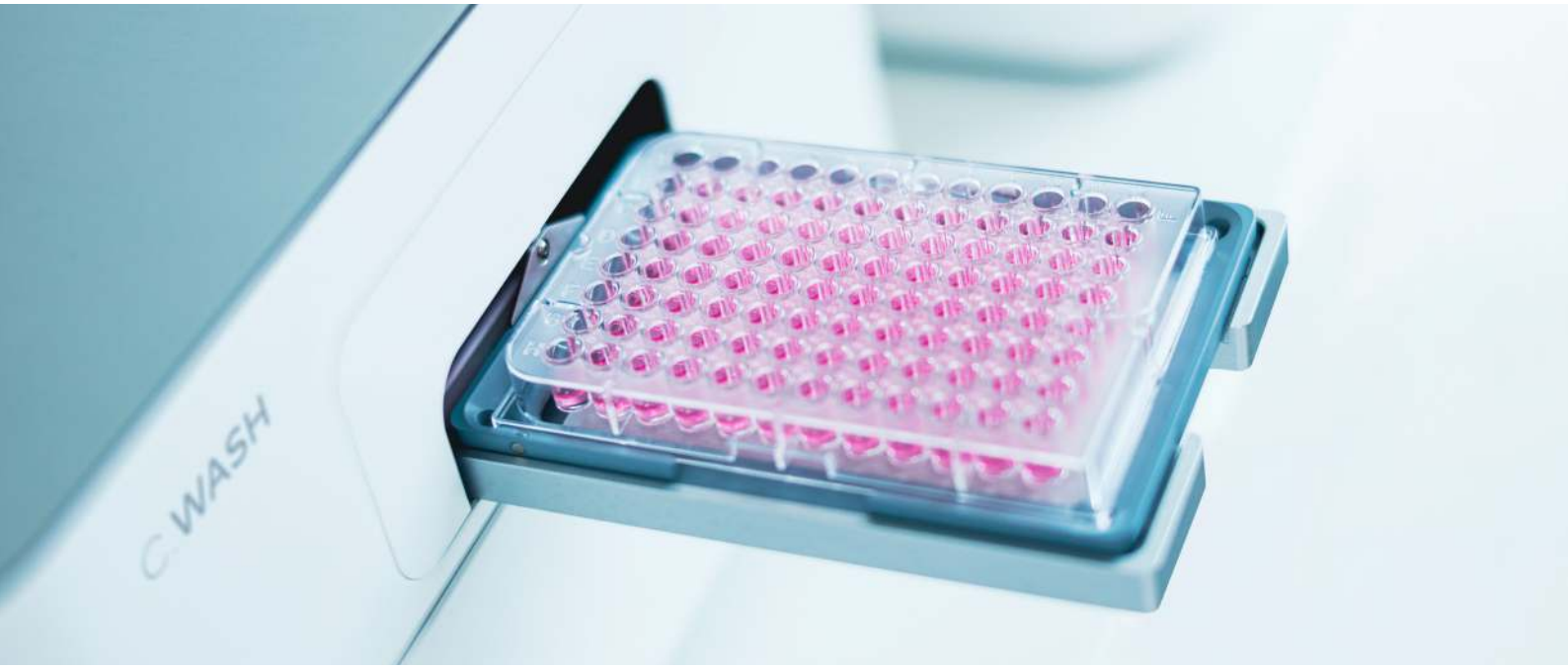


## Introduction

The C.WASH™ plate washer is used to remove liquids from microwell plates by centrifugal forces. The ability to dispense liquids into the plates afterward enables the fully automated and reproducible washing of entire microwell plates in a high-throughput manner.

Here, we determined washing efficiencies achieved by the C.WASH for different rotational speeds in 96-well and 384-well microplate formats. Washing efficiency is measured as the ability to remove media, washing buffer or unbound compounds. Low residual volumes after the washing steps correlate with lower background signals in cell-based assays, ELISA or other immunoassays.

Post-wash and pre-wash fluorescent values of a plate filled with fluorescein solution were compared to determine washing efficiencies. One wash cycle is defined as the removal of liquid in the plate by centrifugal spinning, followed by liquid dispensing. Both steps can be performed using the C.WASH.

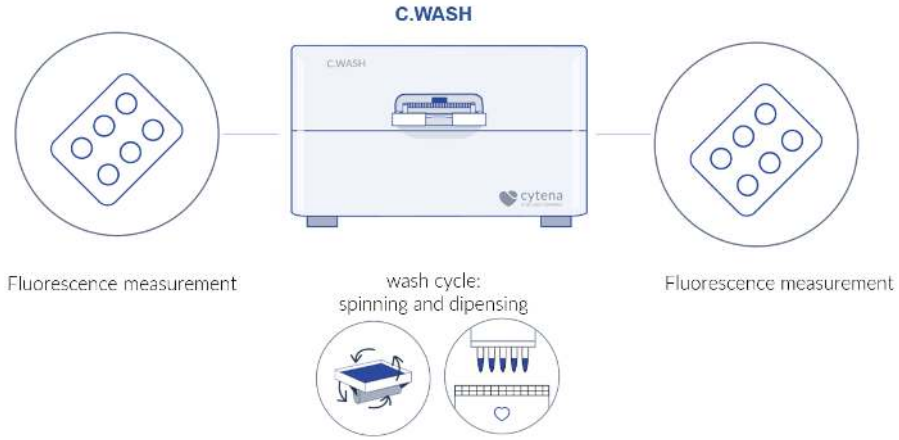


***Figure 1.*** *Workflow for measuring the washing efficiency using the C.WASH centrifugal liquid removal and non-contact dispensing.*

# Materials and methods

## Materials

- Fluorescein (100 µM, dissolved in H2O, CAS-Number 518-47-8)
- Solid black microplates 96-well/384-well, 4titude (4ti-0263/4ti-0264; Brooks Life Sciences)

## Measuring washing efficiencies

For each rotational speed, 96 wells were filled with a 100 µM fluorescein solution (50 µL for 384-well/100 µL for 96-well microplates). Plates were emptied by centrifugal spinning for 5 seconds and subsequently filled with the same volumes of water, dispensed by the C.WASH. The fluorescent signal was measured pre-washing, after one wash cycle and after two wash cycles. Washing efficiencies were calculated as the ratio of fluorescent signal post-washing/pre-washing.

## Quantification of residual volumes

Standard curves for the quantification of remaining volume were dispensed using an I.DOT low volume dispenser. Volumes from 10 nL to 300 nL were dispensed in triplicate. Subsequently 50 µL/100 µL of water were dispensed in all wells using the C.WASH. The resulting standard curve was used to calculate the residual fluorescein values in the experiments.

## Fluorescence measurement

All fluorescence measurements were performed with a Spark Multimode Microplate Reader (Tecan), using fluorescence top-reading.

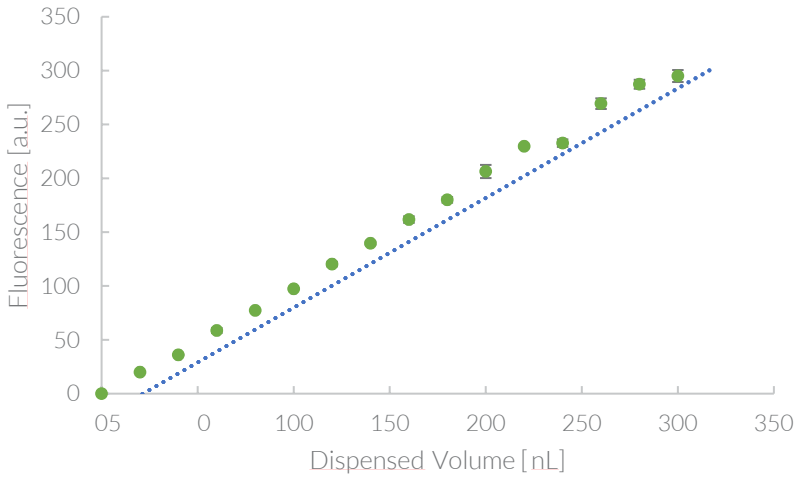


***Figure 2.*** *Standard curve for the quantification of residual volumes, based on fluorescent signal. Measurements were performed in triplicates.*

# Results and discussion

## Washing efficiency

The washing efficiencies for different rotational speeds are shown in **Figure 3A-B.** Even at low rotational speeds (22 xg), 99.5% of the fluorescence was removed after one washing cycle. This efficiency further increases for higher rotational speeds. After a second washing cycle, 99.99% of the fluorescent signal is removed, regardless of the rotational speed. Given these washing efficiencies, using the C.WASH, plate washing protocols can be reduced to a maximum of two washing steps, allowing simplified assay designs and faster results.

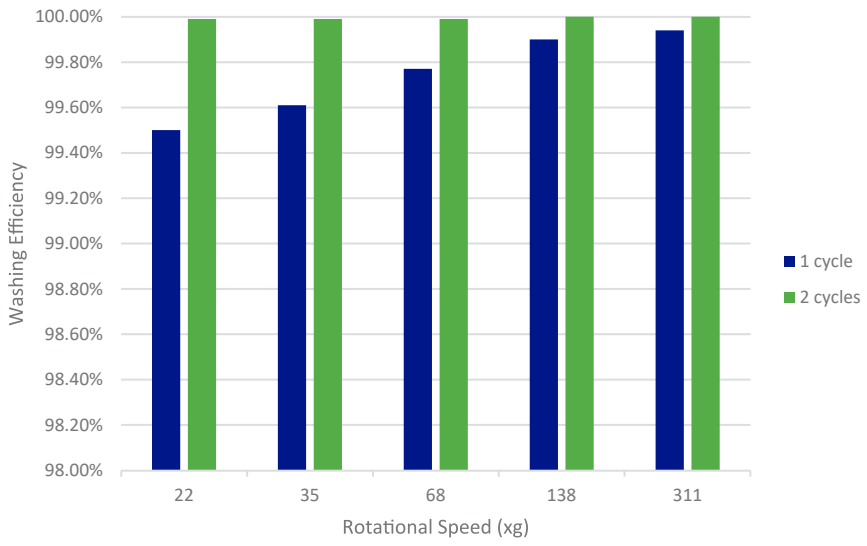


***Figure 3A.*** *Washing efficiencies after one or two washing cycles at different rotational speeds in a 96-well plate. Values shown in the diagram are mean values, deriving from 96 data points.*

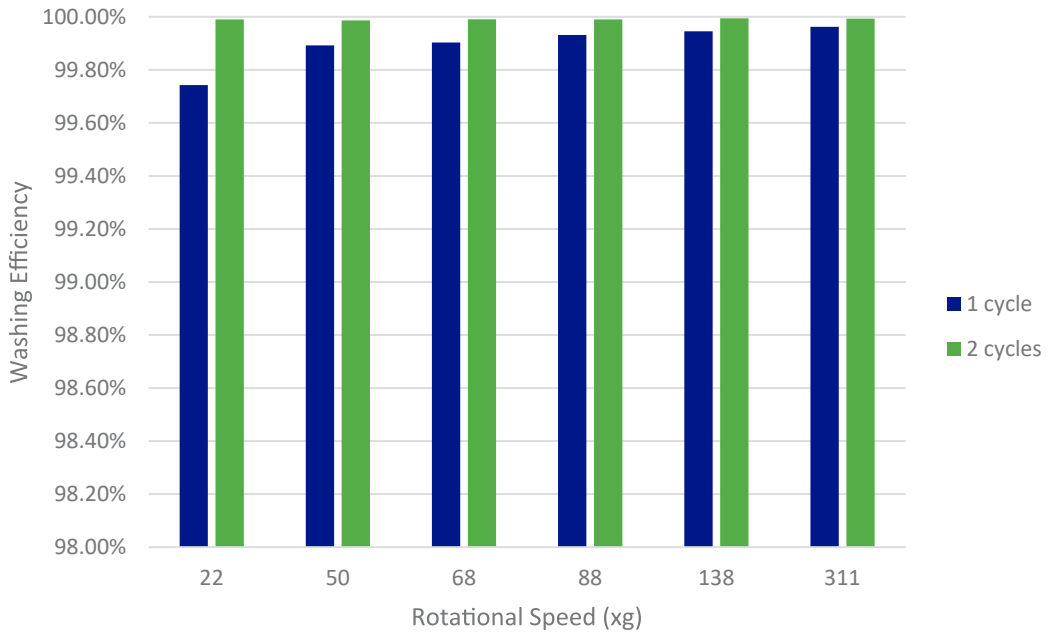


***Figure 3B.*** *Washing efficiencies after one or two washing cycles at different rotational speeds in a 384-well plate. Values shown in the diagram are mean values, deriving from 96 data points.*

# Residual volumes after liquid removal

The remaining volume after one round of spinning are shown in **Figure 4.** Measured volumes are all below 1 µL for all tested parameters, and for 384-well plates even below 250 nL. For the highest rotational speeds, residual volumes are as low as 50 nL. These volumes are more than 10-fold smaller than in needle-based plate washers or with manual plate tapping. Such low residual volumes will lead to reduced background signals in all plate-based assays, leading to better experimental data output.

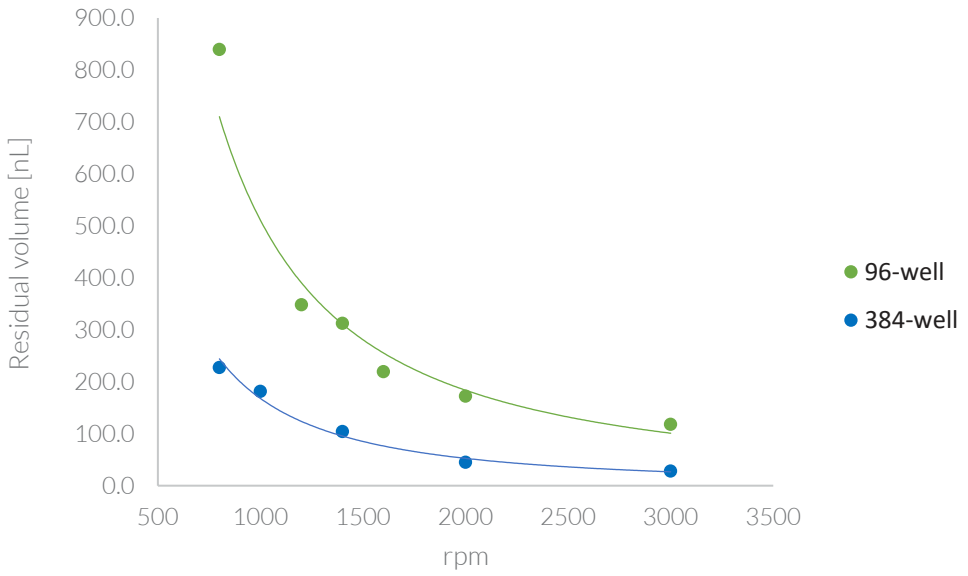


***Figure 4.*** *Residual volumes after spinning at different rpm in 96-well (green) and 384-well (blue) plate format. Values shown in the diagram are mean values, deriving from 96 data points.*

# Conclusion

- The C.WASH achieves very high washing efficiencies after just one wash cycle (>99.5% washing efficiency).
- After two wash cycles, the C.WASH achieved almost complete removal of fluorescent signal (>99.99% washing efficiency).
- Results are highly reproducible due to automation.
- C.WASH highly reduces the number of washing cycles compared to conventional needle-based systems and therefore reduces timelines and reagent consumption.

## CYTENA, A BICO COMPANY

CYTENA spun off from the University of Freiburg, Germany, in 2014 with its patented single-cell dispensing technology. Today, as part of BICO, the world's leading bioconvergence company, CYTENA continues building on that groundbreaking technology to develop high-precision instruments for isolating, dispensing, imaging and handling biological cells. Its award-winning devices are manufactured in Germany and used at prestigious academic and pharmaceutical labs around the world to automate workflows in numerous application areas, including stable cell line development, single-cell omics, high-throughput screening and drug discovery. CYTENA's breakthrough innovations for the lab combine advanced automation, state-of-the-art software engineering and the latest insights in cell biology to maximize efficiencies in the life sciences and create the future of health. Learn more at cytena.com.

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