

# TECHNICAL NOTE

## Precision of the CyBi®-Well vario 384/25 µL Head,

### Example Data of Different Liquids, Pipetting Modes and Disposable Tips with Fluorescence Readout

Katrin Undisz PhD, Heidi Prüfer, and Hanno Hermann PhD, CyBio AG

#### Key words

precision, fluorescence, disposable tips, low volume handling, DMSO, buffer, wet and dry pipetting

#### Summary

In this study the CyBi®-Well vario 384/25 µL Head was used to determine the liquid handling precision with a fluorescence readout over a broad volume range using different pipetting modes, different disposable tips and liquids with different surface tensions. The data show the excellent pipetting performance of the CyBi®-Well vario 384/25 µL Head under various conditions and provide a tool that supports the choice of the right experimental settings and disposable tips for a broad range of customer specific applications.

#### Introduction

The CyBi®-Well vario is well known for fast and precise simultaneous pipetting. Six\* pipetting heads allow the reliable handling of different liquids over a broad volume range. CyBio's in house specification check and quality control is performed with a standardized absorption method (p-Nitrophenol as dye solved in 0.1N NaOH) that is described in detail in every CyBi®-Well or CyBi®-Well vario manual and that is also used to determine the official specification values. In many laboratories fluorescein solutions are used to validate liquid handling devices, because this fluorescent dye is very cost effective, stable for several months and safe to handle due to low toxicity (1). This technical note intends to complete the absorption data by fluorescence data generated with a simple fluorescence intensity measurement. However, it is important to mention, that typically the precision of absorption measurements yields a better resolution than the precision of fluorescence measurements and that simple fluorescence measurements are influenced by a bundle of parameters outside the liquid handling device that can be normalized by a multiwavelength measurement (2,3). In this technical data sheet we show precision data of the CyBi®-Well vario with the 384/25 µL Head using different volumes, different tips, dye solutions with different surface tensions, and different pipetting modes that were determined by a simple fluorescence intensity measurement. These data are example data from typical routine work in our application lab. The data also cover the low volume range below the specification limit and indicate the threshold volumes below the specification limit for which the use of a CyBi®-Well vario Nanoliter Head 384/2.5 µL would be recommended.

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#### References:

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3. Rhode H., Horn A., Schulze M., Cumme G., „Method for Characterizing a Highly Parallelized Liquid Handling Technique Using Microplates and Test Kit for Carrying Out the Method", W= 03/079030 A1, DE 10391021, GB 2403535

\* status march 2007

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## Materials and Reagents

- CyBi®-Well vario with 384/25 µL head (for dye solution) and 384/40 µL head (for buffer)  
25 µL tips (CyBio # OL 2001-25-250)  
10 µL tips (CyBio # OL 2001-25-230)
- 384 well plates PS black (Greiner bio-one # 781 076)
- OmniTrays (Nunc # 140156) as disposable reservoirs
- Fluorescein-Sodium (Standard Fluka # 46960)
- Fluorescein (Reference standard Molecular Probes # F1300)
- PBS (Sigma # P3813)
- DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)
- Adhesive foil (Nunc # 236269)
- PolarStar (BMG Labtechnologies) with filter set 485nm (excitation wavelength) and 520nm (emission wavelength)

## Methods

The precision test was performed in black 384 well plates with a final volume of 40 µL and a final dye concentration of 300 nM. The experimental settings for the different test volumes are described in Tab.1.

test volume [µL]	buffer volume [µL]	fluorescein working solution [µM]
20	20	0.6
10	30	1.2
5	35	2.4
2	38	6
1	39	12
0.5	39.5	24
0.2	39.8	60

Tab.1: Experimental settings to measure the precision of the CyBi®-Well vario 384/25 µL head

To obtain a test solution with low surface tension Fluorescein was dissolved in DMSO, for a test solution with high surface tension Fluorescein-Sodium was dissolved in PBS buffer. The working solutions with the different concentrations were prepared by diluting the dye solution with the highest concentration. All solutions were filtrated before use.

All CyBi®-Well vario pipetting methods were set up in CyBio Control 3.40 according to the following rules:

- one set of new tips per volume and pipetting mode
- piston speed: 100 rpm (characterizes the flow rate of the liquids during aspiration/dispensation;  
= 4 µL/s with a 25 µL head at 100 rpm)
- stage speed: 50 rpm
- priming of tips: 1x with highest tip volume
- break of 1s after every aspiration and dispensing step
- immersion depth of the tips into the liquid = 1 - 2 mm
- final volume 40 µL per well
- final dye concentration 300 nM
- aspiration always with overstroke

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- the first pipetting cycle was always dispensed back into the reservoir
- pipetting of the destination volume as part of the total volume into the test plate
- ejecting the residual volume with maximum overstroke back into the source reservoir or waste
- **dry pipetting** means dispensing the destination volume of the dye solution about 1 mm above the bottom of the dry plate and then touching the droplet by moving the stage up, PBS buffer is used to fill the wells up to the final volume of 40  $\mu\text{L}$
- **wet pipetting** means dispensing the desired volume with tips immersed for about 1 mm into the provided PBS buffer liquid
- immediate sealing of the plates
- shaking of the plates for at least 10 minutes, waiting for at least 30 minutes
- centrifugation of the plates for 2 minutes at 2000 rpm
- readout was performed no earlier than two hours after finishing the pipetting procedure

The precision was calculated as percentage standard deviation (coefficient of variation = CV in %) over a 384-well microplate. Three microplates were prepared per volume and the results were averaged.

## Results and Discussion

In Tab.2 the precision data of the CyBi®-Well vario 384/25  $\mu\text{L}$  head using different volumes, different tips, dye solutions with different surface tensions and different pipetting modes are summarized.

Tips	Test volume	DMSO dry [%CV]	DMSO wet [%CV]	Buffer dry [%CV]	Buffer wet [%CV]
<b>25 <math>\mu\text{L}</math> tips</b>					
	20 $\mu\text{L}$	3.2	2.0	1.8	1.5
	10 $\mu\text{L}$	2.7	2.9	1.7	1.3
	5 $\mu\text{L}$	2.3	2.2	1.7	1.4
	2 $\mu\text{L}$	2.7	2.6	2.7	1.6
	1 $\mu\text{L}$	2.4	2.3	3.2	1.6
	0.5 $\mu\text{L}$	3.7	3.2	13.0	3.0
	0.2 $\mu\text{L}$	n.r.	5.4	(n. r.)	(n.r.)
<b>10 <math>\mu\text{L}</math> tips</b>					
	10 $\mu\text{L}$	4.0	2.3	1.5	2.5
	5 $\mu\text{L}$	3.7	1.9	1.9	2.3
	2 $\mu\text{L}$	3.0	2.6	2.0	2.1
	1 $\mu\text{L}$	4.0	2.6	4.3	2.1
	0.5 $\mu\text{L}$	4.4	3.5	(n. r.)	4.0
	0.2 $\mu\text{L}$	(n. r.)	3.7	(n. r.)	5.2

Tab.2: Overview of precision data (fluorescence readout) that were obtained with the CyBi®-Well vario 384/25  $\mu\text{L}$  head for various volumes with various tips, various liquids and various pipetting modes (n=3), constellations that result in CV values higher than 15% are not recommended (n. r.).

The results indicate, that the CyBi®-Well vario 384/25  $\mu\text{L}$  head allows highly precise high and low volume handling in both, wet-to-wet and wet-to-dry pipetting modes.

At ultra-low volumes less than 1  $\mu\text{L}$  the precision is better in the wet-to-wet pipetting mode (see Fig.1). DMSO solutions can be handled in the wet-to wet mode with the 10  $\mu\text{L}$  tips with precision errors less than 5% CV down to volumes as low as 0.2  $\mu\text{L}$ . For still lower volumes the use of the CyBi®-Well vario Nanoliter Head 384/2.5  $\mu\text{L}$  would be recommended.

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These results strongly indicate the special suitability of the CyBi®-Well vario 384/25 µL head with the 10 µL tips for low volume compound handling in high density plate formats. For this application the precision with the 25 µL tips differs only slightly (see Fig 2).

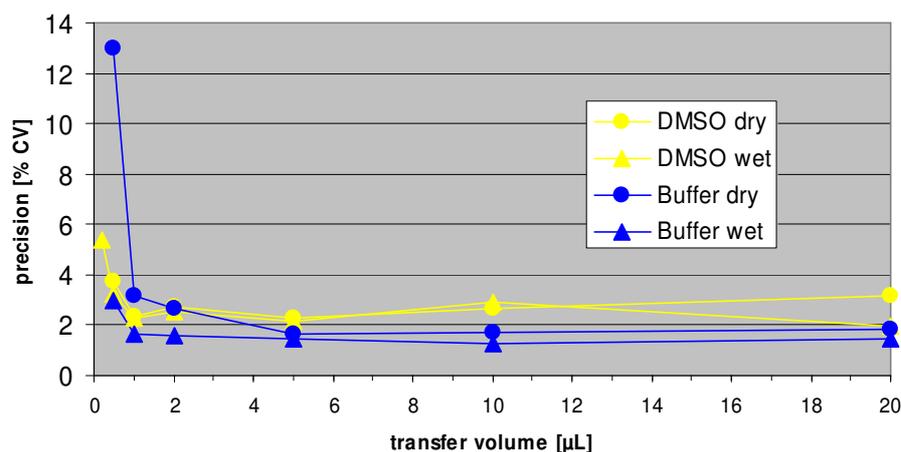


Fig. 1: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 384/25 µL head, comparison of pipetting DMSO and buffer in the wet-to-dry and wet-to-wet mode with 25 µL tips.

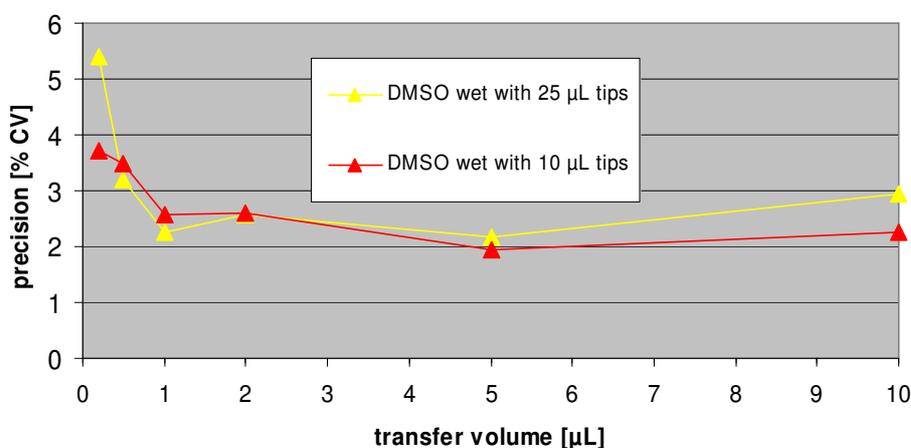


Fig. 2: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 384/25 µL head, comparison of pipetting DMSO in the wet-to-wet mode with 25 µL and 10 µL tips.

Buffer solutions can reliably be handled in the dry mode down to a volume of 1 µL. Precision errors are less than 5 % CV with both 25 µL tips and 10 µL tips. With further decreasing volumes the droplet formation at the tip orifice becomes more and more critical due to the high surface tension (that has to be overcome) and the precision error increases (for 0.5 µL to 13 % CV with 25 µL tips and to more than 15 % CV with 10 µL tips, see Tab.2).

Further, the quality of the microplates, especially the flatness of the plate bottom, is essential for the outcome of wet-to-dry liquid transfers. CyBi®-Well and CyBi®-Well vario allow a vertical adjustment of the stage height, in steps of 1/10 mm, for touching the tips to the plate bottom in order to reliably deposit a droplet. Often 1/10 mm stage height difference is enough to cross the rubicon between good precision data and not acceptable results.