

# TECHNICAL NOTE

## Precision of the CyBi®-Well vario 96/250 µL Head,

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

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#### Key words

precision, fluorescence, disposable tips, deep well plates, DMSO, buffer, wet and dry pipetting

#### Summary

Preferred application fields of the CyBi®-Well vario 96/250 µL pipetting head are the parallel reagent transfer of higher volumes and all liquid handling procedures in deep well plates.

In this study the CyBi®-Well vario 96/250 µL pipetting head was used to determine the liquid handling precision with a fluorescence readout over a broad volume range using different pipetting modes, shallow well as well as deep well tips and different liquids. The data show the excellent pipetting performance of the CyBi®-Well vario 96/250 µL pipetting head and also indicate the threshold volumes under various conditions thus supporting the choice of the right pipetting head, the optimal experimental settings and the appropriate 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 (low toxicity) (1). This technical note intends to complete the absorption data by fluorescence data generated with a simple fluorescence intensity measurement. We show precision data of the CyBi®-Well vario 96/250 µL head using different volumes, shallow well as well as deep well tips, different pipetting modes and different dye solutions.

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).

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#### References:

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For our precision measurement we used an aqueous buffer solution as example for a protic solvent and DMSO as important polar aprotic solvent for different application fields. Both liquids differ in their physicochemical characters which influence the solvent properties as well as the liquid transfer behavior. The surface tension (high for water: 72.75 mN/m; low for DMSO: 25 mN/m, at 20°C) is responsible for the interface behavior of the liquids (adhesion, adsorption, wetting) on the surface of tips and plates. The viscosity (low for water: 1.0 mPa s; high for DMSO: 19.96 mPa s, at 20°C) mainly influences the droplet formation and fluidity in the liquid handling system.

These data of this study are example data from typical routine work in our application lab. The data also indicate the threshold volumes below the specification limit for which the use of a CyBi®-Well vario 96/25 µL head would be recommended.

## Materials and Reagents

- CyBi®-Well vario with 96/250 µL head (for buffer and dye solution)
  - 250 µL shallow well tips (CyBio # OL 2001-25-300) (46 mm tip length)
  - 250 µL deep well tips (CyBio # OL 2001-25-350) (68 mm tip length)
- 96 well plates PS black (Greiner bio-one # 655 076)
- 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 96 well plates with a final volume of 200 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]
100	100.0	0.6
50	150.0	1.2
10	190.0	6
5	195.0	12
2	198.0	30
1	199.0	60

Tab.1: Experimental settings to measure the precision of the CyBi®-Well vario 96/250 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.

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All CyBi®-Well vario 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; = 41 $\mu$ L/s with a 250 $\mu$ L head and 4  $\mu$ L/s with a 25  $\mu$ L head at 100 rpm, respectively)
- stage speed: 50 rpm
- priming of tips: at least 1x with highest tip volume
- break of 1s after every aspiration and dispensing step
- immersion depth 1 - 2 mm
- final volume 200 $\mu$ L per well
- final dye concentration 300nM
- aspiration always with overstroke
- dispensing back of the first pipetting cycle into the reservoir
- pipetting of the target volume as part of the total volume into the test plate
- **dry pipetting** means dispensing the desired 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 200  $\mu$ L
- **wet pipetting** means dispensing the desired volume with tips immersed for about 1mm into the provided PBS buffer liquid
- ejecting the residual volume with maximum overstroke back into the source reservoir or waste
- 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 not earlier than two hours after finishing the pipetting procedure.

The precision data were calculated as percentaged standard deviation (coefficient of variation = CV in %) over a 96-well microplate. Three microplates were prepared per volume and the results were averaged. The results can be reproduced when these methodical details are considered.

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## Results and Discussion

In Tab.2 the precision data of the CyBi®-Well vario 96/250 µL 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>250 µL tips (shallow well, 46 mm)</b>					
	100 µL	1.5	1.4	1.7	1.4
	50 µL	1.4	1.7	1.7	1.6
	10 µL	3.0	2.0	3.9	1.6
	5 µL	2.3	1.5	5.7	1.6
	2 µL	3.6	1.4	n. r.	n. r.
	1 µL	n. r.	1.8	n. r.	n. r.
<b>250 µL tips (deep well, 68 mm)</b>					
	100 µL	1.9	1.3	1.5	1.5
	50 µL	2.0	1.3	1.7	1.7
	10 µL	3.0	1.3	3.6	1.4
	5 µL	4.8	1.3	6.4	1.5
	2 µL	7.0	1.5	n. r.	n. r.
	1 µL	n. r.	2.5	n. r.	n. r.

Tab.2: Overview of precision data that were obtained with the CyBi®-Well vario 96/250 µL 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 96/250 µL head allows highly precise liquid handling in both, wet-to-wet and wet-to-dry pipetting modes.

As expected, the precision data are somewhat better in the wet mode than in the dry mode (see Fig.1). Because of the lower internal distance between liquid surface and pistons the results with the shallow well tips, especially in the low volume range, are still better than those obtained with the deep well tips (see Fig.2). For all standard applications in shallow well plates the shallow well tips are the best choice, the deep well tips are recommended for all applications dealing with deep well plates or deep well reservoirs.

In both pipetting modes and with both tip versions the precise handling of buffer solutions with the CyBi®-Well vario 96/250 µL head is possible down to 5 µL. DMSO can be handled precisely down to 2 µL in the wet-to-dry mode and even down to 1 µL in the wet-to-wet mode.

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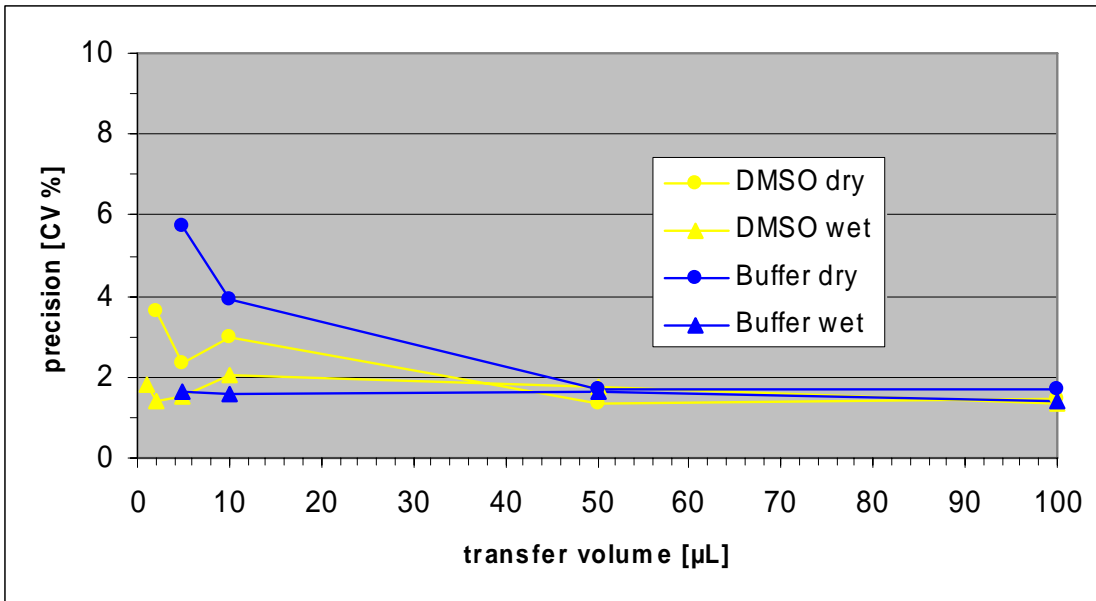


Fig.1: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 96/250µL head, comparison of pipetting DMSO and buffer in the dry and wet mode with 250 µL shallow well tips (46 mm).

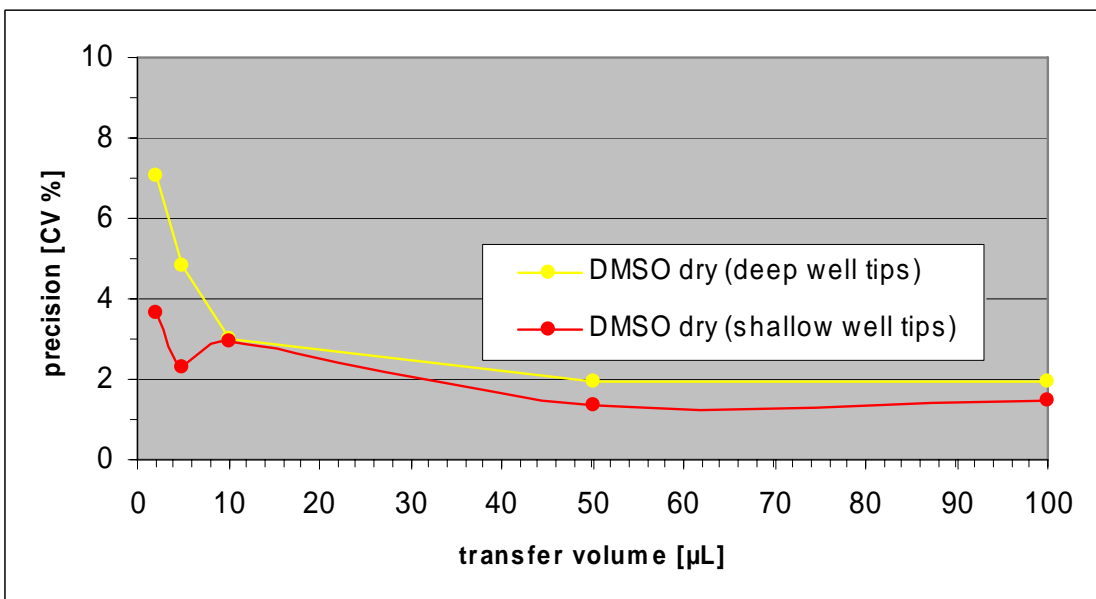


Fig.2: Precision data (fluorescence readout) obtained with the CyBi®-Well vario 96/250 µL head, comparison of 250 µL shallow well tips and 250 µL deep well tips, by pipetting DMSO in the dry mode.

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. With decreasing volumes the droplet formation at the tip orifice becomes more and more critical and the precision error increases to CV values more than 15.0 % (see Tab.2) because more and more wells stay empty. Therefore for volumes below the indicated threshold levels the use of a CyBi®-Well vario 96/25 µL head is recommended.