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Precision of Different 96-Capillary Type Magazines of the CyBi®-Well vario Capillary Head - Example Data

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Introduction

The Capillary Head of the CyBi®-Well vario is an exchangeable pipetting head based on the principle of capillary action. 96 or 384 capillaries with defined liquid capacities are filled simultaneously by capillary forces via dipping them into the liquid of a source plate. Figure 1 shows the Capillary Head with an inserted 96-capillary magazine and 50 nl capillaries as well as a summary of all available capillary types.

Color	Volume
White	25 nl
Red	50 nl
Yellow	100 nl
Green	250 nl
Blue	500 nl
Orange	750 nl
Violet	1000 nl



The dispensing is done by applying an air pressure pulse on all capillaries at the same time resulting in a parallel blow out of the capillaries into the empty wells of a destination plate. The action of the Capillary Head includes the proven and convenient features of the CyBi®-Well vario family including the plate handling system. It is a very robust, precise, and accurate tool especially for wet-to-dry non-contact compound handling in the nanoliter range.

In this Technical Note precision data are summarized which were measured during CyBio's in-house quality control check of different 96-capillary type magazines.



Figure 1: The CyBi®-Well vario Capillary Head with inserted 96 capillary magazine and 50 nl capillaries as well as a summary of the different capillary types.

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Materials

Devices

- » CyBi®-Well vario with Capillary Head and 96/250 µl head (for addition of 0.1N NaOH)
- » CyBi®-Composer version 2.13 or higher
 - Capillary magazine 96/25 nl
 - Capillary magazine 96/50 nl
 - Capillary magazine 96/100 nl
 - Capillary magazine 96/250 nl
- » BioTek ELx800 microplate absorbance reader with 405 nm filter

Consumables

- » 250 µl tips (CyBio #OL 3800-25-559-N)
- » 96 well plates transparent flat bottom (Greiner bio-one # 655 101)
- » OmniTrays (Nunc # 140156) as reservoirs
- » Adhesive foil (Nunc # 236269)

Reagents

- » p-Nitrophenol (p-NP, Sigma # 1048)
- » 0.1 N NaOH (Sigma # 71689)
- » DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)

Methods

The Adjustment of the Optimal Air Pressure Parameters

On the part of the customer an oil-free air pressure control unit has to be provided with a maximum allowable working pressure of 5 bar (73 psi). The real working pressure has to be adjusted via the corresponding air pressure control unit to 1.72 bar (25 psi). The maintenance instructions for the air pressure control unit have to be considered.

The Determination of the Precision

The precision of the liquid transfer with the Capillary Head was determined using p-Nitrophenol solved in DMSO as test solution, filling up the plates with 0.1 N NaOH, and measuring of the absorbance at 405 nm. Table 1 summarizes the recommended p-Nitrophenol concentrations for the different 96-well capillary type magazines.

The final p-Nitrophenol concentration in all wells of a microplate always should be about 120 µM to give an absorption signal in the linear range.

The detailed steps of the procedure are:

1. Wash capillaries 10x (in case of first usage 20x)
2. At least 3 dry blow out steps with an air pressure pulse
3. Transfer of the appropriate p-Nitrophenol test solution into empty transparent microplates (about 4 mm dipping depth of the capillaries into the test solution, 8 s time for aspiration, 1.5 mm dispensing height above the floor of the test plate)
4. Fill up 0.1 N NaOH (200 µl/well in 96-well microplates)
5. Seal the microplates
6. Shake the microplates at 1000 rpm (switch on slowly!) for 10 minutes
7. Centrifuge at 2000 rpm for 2 minutes
8. Wait for half an hour
9. Remove sealing foil and measure absorbance at 405 nm
10. Calculate the precision error

Table 1: Recommended p-Nitrophenol test solution concentrations to determine the precision of the different 96-well capillary type magazines.

Volume of p-Nitrophenol test solution	p-Nitrophenol test solution in DMSO for a 96-well capillary magazine (200 µl final volume per well)
25 nl	960 mM
50 nl	480 mM
100 nl	240 mM
250 nl	96 mM
500 nl	48 mM
750 nl	36 mM
1000 nl	24 mM

Following the dispensing of the desired test solution, the wells were filled up with 200 µl 0.1 N NaOH per 96-well of a measurement plate, and the

absorbance was measured following shaking and waiting for homogeneous distribution of the dye in the wells.

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The Capillary Cleaning Routine

Before and following every use, the capillaries have to be washed carefully. An effective cleaning routine for the active Capillary Wash Station 96 (CWS 96) was set up in the CyBio® Composer Software with the parameters which are summarized in Table 2. Before starting the cleaning procedure, the active CWS has to be filled with washing liquid (deionized water or water with 10 % DMSO is recommended, higher DMSO concentration are also possible), until a constant liquid level is reached.

For draining individual pumps for the wash liquid and the waste liquid have to be used. Connecting the waste tube of the CWS with an external vacuum system with integrated waste collection flask also is possible.

A final drying step with ethanol from a reservoir is recommended before a capillary magazine is changed or placed back into the original storage box. The dispensing can be done easily into an empty plate or into a disposable reservoir with a filter paper.

Table 2: Recommended parameters to set up an automated cleaning routine for the CWS 96 in CyBio Composer.

Parameter	
Stage speed	50 - 70 rpm
Final speed of the source pump	≤ 20 rpm
Final speed of the drain pump (use mode "characteristic curve")	≥ 300 rpm
Dipping depth into the wash liquid in the CWS (corresponding to the glass part of the capillaries)	≥ 2 mm
Time for filling of the capillaries at the aspirate position of the CWS	8 s
Dispensing with a blow out into the chimneys of the CWS at the dispense position, tips immersed as deep as possible	1 pulse
Washing cycles	10

Results and Discussion

In Table 3 the precision data which were determined for different 96 capillary magazines with different capillary types are summarized. Three measurements were performed with every capillary type magazine.

In the specification the precision error of all types of capillary magazines is guaranteed to be less than 10 %. For higher capillary volumes also a higher precision can be expected.

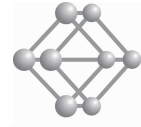
In our experiments the average precision error of 96-capillary magazines with 25 nl capillaries was about 8 %, with 50 nl capillaries about 5 %, with 100 nl capillaries about 3 %, and with 250 nl capillaries less than 2%, respectively.

These data are in agreement with the precision results of different 384 capillary type magazines (1). The mean values per magazine individually reflect the quality of the capillaries of one specific magazine. Multiple measurements with one capillary magazine show the high reproducibility of the results.

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Table 3: Summary of precision data which were determined for different 96 capillary magazines with different capillary types (n=3 per magazine). The colors of the rows are in accordance with the capillary colors.

Capillary Volume [nl]	Magazine N°	p-NP Test Solution [mM]	Single Measurements CV [%]	Mean per Magazine CV [%]	Mean per Capillary Type CV [%]
25	1	960	8.2		
25	1	960	8.1		
25	1	960	8.1	8.1	
25	2	960	9.3		
25	2	960	8.0		
25	2	960	9.3	8.8	
25	3	960	6.6		
25	3	960	7.3		
25	3	96	6.8	6.9	
25	4	960	8.6		
25	4	960	8.3		
25	4	960	7.8	8.2	8.0
50	1	480	5.8		
50	1	480	5.3		
50	1	480	6.2	5.3	
50	2	480	4.9		
50	2	480	5.7		
50	2	480	5.7	5.4	
50	3	480	3.8		
50	3	480	4.0		
50	3	480	4.6	4.1	5.1
100	1	240	3.8		
100	1	240	4.2		
100	1	240	3.8	3.9	
100	2	240	3.8		
100	2	240	3.3		
100	2	240	3.9	3.7	
100	3	240	4.0		
100	3	240	3.0		
100	3	240	3.4	3.5	
100	4	240	2.7		
100	4	240	3.4		
100	4	240	3.4	3.2	
100	5	240	2.7		
100	5	240	2.6		
100	5	240	2.3	2.5	3.0
250	1	96	1.8		
250	1	96	1.7		
250	1	96	1.8	1.8	
250	2	96	1.9		
250	2	96	1.8		
250	2	96	1.7	1.8	1.8



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References

1. Undisz, K., Prüfer H., and Hermann H. (2009); "Precision of Different 384-Capillary Type Magazines of the CyBi®-Well vario Capillary Head - Example Data", Technical Note, www.cybio-ag.com

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