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TECHNICAL NOTE

Precision of the new Ceramic Type Capillary Magazines 384 of the CyBi®-Well vario Capillary Head - Example Data

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Key words
precision, absorbance, capillary technology, ceramic capillaries, low volume compound handling, DMSO, dry pipetting

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. The first generation of capillaries was made from small polyimide coated glass tubes of different inner diameter and length embedded in a polypropylene shaft. The second generation is now available and consists of monolithic zirconium oxide capillaries embedded in a PEEK shaft. Zirconium oxide is chemically inert and has a higher stiffness value compared to glass. The PEEK shaft is also more vertically rigid than the thermoplastic shaft which is advantageous especially for handling of 1536 well microplates. In both types of capillary magazines single capillaries are exchangeable. Fig. 1 shows the new ceramic capillaries with the established color code.

Fig. 1: Different new ceramic type capillaries with PEEK shaft

Color	Volume
green	250 nl
yellow	100 nl
red	50 nl

The dispensing is carried out 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 base unit of the CyBi®-Well vario family including the capillary wash station and the plate handling system. Stackers can be equipped with a sensor set to detect misaligned microplates automatically. The capillary technology is very robust, precise and accurate and was especially designed for wet-to-dry non-contact compound handling in the nanoliter range.

In this Technical Note precision data is summarized which was generated with the new ceramic type capillary magazines during CyBio´s in-house quality control check.

Materials and Reagents

- CyBi®-Well vario with Capillary Head and 384/60 µl Head (for addition of 0.1N NaOH)
  - 60 µl tips (CyBio # OL 3800-25-515-N)
  - Ceramic capillary magazine 384/50 nl
  - Ceramic capillary magazine 384/100 nl
  - Ceramic capillary magazine 384/250 nl
- CyBi®-Composer version 2.53
- 384 well plates transparent flat bottom (Greiner bio-one # 781 101)
- OmniTrays (Nunc # 140156) as disposable reservoirs
- p-Nitrophenol (p-NP, Sigma # 1048)
- 0.1 N NaOH (Sigma # 71689)
- DMSO (SeccoSolv Merck Darmstadt # 1.02931.1000)
- Adhesive foil (Nunc # 236269)
- TECAN SPECTRAFluor PLUS microplate reader with 405 nm filter
- Laboratory centrifuge SIGMA 6K15 with microplate rotor
- Bio Shake iQ (Q.Instruments QUANTIFOL Instruments GmbH)

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Methods

The Adjustment of the Optimal Air Pressure Parameters

An oil-free air pressure control unit was used with a maximum working pressure of 5 bar (73 psi). The real working pressure was adjusted via the corresponding air pressure control unit to 1.0 bar (15 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 new ceramic type capillary magazines 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. Tab. 1 summarizes the recommended p-Nitrophenol concentrations for the different 384 well ceramic type capillary magazines.

Volume of p-Nitrophenol test solution	p-Nitrophenol test solution in DMSO for a 384 well capillary magazine (50 µl final volume per well)
50 nl	120 mM
100 nl	60 mM
250 nl	24 mM

Tab. 1: Recommended p-Nitrophenol test solution concentrations to determine the precision of the different 384 well ceramic 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 CyBio´s in-house quality control procedure are:

1. wash capillaries 5 x, at least 2 dry blow out steps with an air pressure pulse and vacuum evacuation
2. transfer of the appropriate p-Nitrophenol test solution into empty transparent microplates (about 2 mm dipping depth of the capillaries into the test solution, 6 s time for aspiration, 1 - 1.5 mm dispensing height above the floor of the test plate)
3. fill up 0.1 N NaOH (50 µl/well in 384 well microplates)
4. seal the microplates
5. shake the microplates at 2000 rpm for 2 minutes
6. centrifuge at 2000 rpm for 2 minutes
7. wait for half an hour
8. remove sealing foil and measure absorbance at 405 nm
9. calculate the precision error

Following the dispensing of the desired test solution, the wells were filled up with 50 µl 0.1 N NaOH per 384-well of a measurement plate, and the absorbance was measured following shaking and waiting for homogeneous distribution of the dye in the wells.

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 384 (CWS 384) was set up in the CyBio® Composer Software with the parameters which are summarized in Tab.2.

Parameter	
Stage speed	50 - 70 rpm
Final speed of the source pump for prefilling the trough	50 rpm
Final speed of the source pump for washing capillaries	≤ 130 rpm
Final speed of the drain pump	200 rpm
Dipping depth into the wash liquid in the CWS (corresponding to the ceramic part of the capillaries)	≤ 2 mm
Time for filling of the capillaries at the aspirate position of the CWS	6 s
Dispensing with a blow out into the sleeves of the CWS at the dispense position, tips immersed as deep as possible	2 pulses
Vacuum evacuation time	0.5 s
Washing cycles	5

Tab. 2: Recommended parameters to set up an automated cleaning routine for the CWS 384 in CyBio® Composer

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Results and Discussion

In Tab 3. the precision data which were determined for different ceramic type capillary magazines 384 are summarized. Two different magazines were tested per ceramic capillary size and three measurements were performed with each magazine.

capillary volume [nl]	magazine N°	p-NP test solution [mM]	single measurements CV [%]	mean per magazine CV [%]	mean per capillary type CV [%]
50	1	120	2.0	2.2	2.3
50	1	120	2.3		
50	1	120	2.2		
50	2	120	2.7	2.4	
50	2	120	2.3		
50	2	120	2.3		
100	1	60	1.5	1.4	1.4
100	1	60	1.3		
100	1	60	1.4		
100	2	60	1.3	1.3	
100	2	60	1.3		
100	2	60	1.4		
250	1	24	1.2	1.2	1.4
250	1	24	1.2		
250	1	24	1.2		
250	2	24	1.6	1.6	
250	2	24	1.5		
250	2	24	1.6		

Tab. 3: Summary of precision data which were determined for capillary magazines 384 with different ceramic type capillaries (n=3 per magazine).

In our experiments the average precision error of 384 capillary magazines with 50 nl ceramic capillaries was less than 3 % and of that with 100 nl and 250 nl ceramic capillaries was less than 2 %, respectively. These data are comparable or even better than the results which were achieved with glass type capillary magazines (1).

References

1. Undisz K., Pruefer H. and Hermann H. (2008); "Precision of different 384-Capillary Type Magazines of the CyBi®-Well vario Capillary Head – Example data"; TechNote CyBio AG; www.cybio-ag.com

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