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“How To Use” Guide for the Capillary Head of the CyBi®-Well vario

System Overview

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 summarizes the different capillary types.

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 base unit of the CyBi®-Well vario family including the plate handling system. It is a very robust, precise and accurate tool especially for compound handling in the nanoliter range. Two types of active Capillary Wash Stations in the format 96 as well as 384 were developed to enable a simple and effective cleaning of the capillaries.



Figure 1: The different capillary types of the CyBi®-Well vario Capillary Head with colour code and corresponding volume

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). At these conditions the air consumption of the vacuum device is about 20 l/min at standard atmospheric conditions. The maintenance instructions for the air pressure control unit have to be considered.

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 (CWS) should be set up in the CyBio® Composer Software, we recommend the method parameters which are summarized in Table 1. Before starting the cleaning procedure the trough of the active CWS has to be prefilled with reduced source pump speed (see Table 1) until a constant liquid level is reached. Deionized water with a suitable detergent or water with 10% DMSO is recommended as washing liquid, higher DMSO concentration are possible.

The active Capillary Wash Station is equipped with a wash solution inlet (green), a waste outlet (red) and a vacuum port (black). The final pump speed of the drain pump should be at least 50 rpm higher than that of the source pump speed, recommended default values are shown in Table 1. Depending on the volume of the capillaries and on the number of washing cycles the contaminated washing liquid has to be soaked away from time to time to avoid splashing. This can be realized by a short switching on of the vacuum system following several blow-outs. The waste is collected in an adapted vacuum resistant flask which is available in different sizes.

Method Parameters

Final speed of the drain pump	180 rpm
Final speed of the source pump for prefilling the trough	50 rpm
Final speed of the source pump for washing the capillaries	130 rpm
Stage speed	50 - 70 rpm
Dipping depth into the wash liquid at the aspirate position between the sleeves (corresponding to the glass part of the capillaries)	about 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 (dipping of the capillaries into the sleeves as deep as possible)	at least 2 pulses
Washing cycles	5-10
Time between switching on and switching off vacuum aspiration	0.5 s

Table 1: Recommended method parameters to set up an automated cleaning routine in CyBio® Composer Software

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 onto a filter paper.

Intensive Capillary Cleaning

After defined intervals of routine use or in the case of clogging, an intensive capillary cleaning procedure alternately with a basic and an acidic cleaning solution should be performed. The following example cleaning solutions are recommended:

Alkaline cleaning solution

10 % RBS 35 Concentrate or 0.1 N NaOH (Carl Roth GmbH Karlsruhe, # 9234.2)

Acidic cleaning solution

5 % citric acid or a usual liquid cleaner for coffee machines according to the instructions of the manufacturer

For intensive capillary cleaning the usual cleaning routine with wash liquid should be repeated with alkaline cleaning solution, wash liquid, acidic cleaning solution and again with wash liquid. A final cleaning routine with ethanol is recommended before storage to accelerate drying. In the case of persistent clogging the capillaries may remain immersed for up to 30 s per washing cycle. If following this procedure the values of single capillaries still differ significantly from the average these capillaries should be replaced by new ones.

Maintenance of the Capillary Wash Station

After defined intervals of routine use a maintenance of the Capillary Wash Station is recommended to clean the sleeves and the waste disposal level. For this purpose the inflow holes have to be blocked with the enclosed sealing tool (or a lab tape) and the tube of the wash solution inlet has to be connected with the vacuum port. Then wash liquid has to be pumped through the Capillary Wash Station. The implementation of breaks may im-

prove the cleaning effect. To empty the sleeves and the waste disposal level the orientation of the tube guide in the peristaltic pump has to be changed. It is recommended to repeat this cleaning procedure with ethanol to improve the cleaning effect and to accelerate drying. Finally the original tube connection has to be rebuilt, the sealing tool has to be removed and the remaining liquid should be evacuated using the vacuum system.

The Determination of the Liquid Transfer Precision of the CyBi®-Well vario Capillary Head

For all height adjustments during method set up the use of an adjustment magazine is recommended which is available for capillary magazines 96 and 384.

The precision of the liquid transfer with the Capillary Head can be 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 2 summarizes the p-Nitrophenol concentrations for the different capillary types in dependence on the plate format. The final p-Nitrophenol concentration in all wells of a microplate always should be about 120 µM to give an absorbance signal in the linear range. Following the dispensing of the desired test solution, the wells are filled up with 50 µl (384 well plate format) or 200 µl (96 well plate format) 0.1 N NaOH, respectively, and the absorbance is measured at 405 nm following shaking and waiting for homogeneous distribution of the dye in the wells. Further methodical details as well as typical experimental results are described in CyBio Technical Notes (1, 2).

Volume	p-Nitrophenol test solution in DMSO for a 384 well capillary magazine (50 µl final volume)	p-Nitrophenol test solution in DMSO for a 96 well capillary magazine (200 µl final volume)
25 nl	240 mM	960 mM
50 nl	120 mM	480 mM
100 nl	60 mM	240 mM
250 nl	24 mM	96 mM
500 nl	12 mM	48 mM
750 nl	8 mM	32 mM
1000 nl	6 mM	24 mM

Table 2: Recommended p-Nitrophenol test solution concentrations to determine the precision of the different capillary types in dependence on the capillary magazine format

The detailed steps of the procedure are:

1. Wash capillaries 10x (in case of first usage 20x)
2. At least 2 dry blow out steps with an air pressure pulse
3. 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, 1.0 – 1.5 mm dispensing height above the floor of the test plate)
4. Fill up 0.1 N NaOH (50 µl/well in 384 well microplates, 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

If this method is preformed carefully the precision error of all magazines is specified to be less than 10%. Especially with higher volumes the expected CV values get better.

Typical experimental precision error values could be:
 25 nl: CV < 9%
 100 nl: CV < 3%
 250 nl: CV < 2%

The Storage of the Capillary Magazines

The cleaned capillary magazines should be removed from the Capillary Head and stored exclusively in the original storage box. Any mechanical contact of the capillaries with solid parts of the instrument or the storage box has to be avoided carefully.

Exchange of Single Capillaries

If the values of single capillaries differ significantly from the average, these capillaries should be gently unscrewed and replaced by new ones. This procedure is described in detail in the CyBi®-Well vario manual at point 9.2.5.

References

1. Undisz K. et. al. (2009), "Precision of Different 96-Capillary Type Magazines of the CyBi®-Well vario Capillary Head - Example Data", TechNote Cybio AG, www.cybio-ag.com
2. Undisz K. et. al. (2009), "Precision of Different 384-Capillary Type Magazines of the CyBi®-Well vario Capillary Head - Example Data", TechNote Cybio AG, www.cybio-ag.com

CyBio AG

Goeschwitzer Strasse 40
07745 Jena
Germany
Tel +49.3641.351 0
Fax +49.3641.351 409

info@cybio-ag.com
www.cybio-ag.com

CyBio US, Inc.

500 West Cummings Park
Suite 1800
Woburn, MA 01801, USA
Tel +1.781.3 76 98 99
Fax +1.781.3 76 98 97

CyBio Northern Europe Ltd.

8 James Whatman Court, Turkey Mill
Ashford Road, Maidstone
Kent ME 14 5SS, UK
Tel +44.16 22 66 21 18
Fax +44.16 22 66 41 57

CyBio
An Analytik Jena Company