

TECHNICAL NOTE

Precision of the 96-Channel Head of the CyBi®-CrystalCreator

Example Data of Precise Liquid Handling in the Low Volume Range Using Different Pipetting Modes

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Key words

precision, low volume liquid handling, absorbance, disposable tips, DMSO, wet and dry pipetting, protein crystallography

Abstract

The CyBi®-CrystalCreator is a workstation for protein crystallography that involves a 96-channel parallel pipetting head and a special 8-channel head, both with a maximal volume of 25 µL. A 10 position circular carriage is used as a working table. In this technical note we characterize the precision of the 96-channel head of the CyBi®-CrystalCreator in the low volume range with a standardized absorption method using different volumes and pipetting modes. The data demonstrate the excellent pipetting performance of the 96-channel head of the CyBi®-CrystalCreator under various conditions.

Introduction

The CyBi®-CrystalCreator is the ideal workstation for the efficient and reliable automation of various protein crystallization methods as sitting drop, hanging drop and microbatch. The combination of a 96- and an 8-channel pipettor allows flexible, fast and precise liquid handling, while the circular 10-position carriage assures sufficient space for crystallization plates and reagent reservoirs. For the transfer of different protein solutions and crystallization liquids into crystallization plates the precise transfer of volumes as low as 0.1 µL is essential.

In this technical note we show precision data in the low volume range of the 96-channel head of the CyBi®-CrystalCreator using different volumes as well as different pipetting modes. The precision was determined using a standardized absorption method. The data show the excellent pipetting performance and also indicate the optimal experimental settings for low volume liquid handling applications such as protein crystallography experiments.

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Materials and Reagents

- CyBi®-Well vario with a 96/250 µL head (for pipetting of 0.1 N NaOH simultaneously to microplates)
250 µL shallow well tips (46 mm tip length, CyBio # OL 2001-25-300)
- 96-channel head of the CyBi®-CrystalCreator with a maximal volume of 25 µL and a 10-position circular carriage
25 µL tips (CyBio #OL 2001-25-250)
- 96 well microplates PS transparent (Greiner bio-one #655 101)
- Height adapter (CyBio #OL 3397-25-110)
- OmniTrays as disposable reservoirs (Nunc #140156)
- Tolerance mat (CyBio #OL 3518-28-940)
- p-Nitrophenol (p-NP, Sigma #104-8)
- NaOH (Roth Karlsruhe #6771.3)
- DMSO (Merck Darmstadt #1.02931.1000)
- Adhesive foil (Nunc #236269)
- Absorption reader ELX800 (BioTek)
- Centrifuge 6K15 with Rotor No. 11150 (Sigma)
- IKA®-Shaker MTS4 (IKA®-Werke GmbH & Co.KG)

Methods

The precision test was performed according to the standardized CyBio in house specification check and quality control procedure that is described in detail in the user manuals. In brief, transparent 96 well microplates were used for absorption measurements with a final volume of 200 µL and a final p-nitrophenol (p-NP) concentration of 60 µM per well. For this purpose, different p-NP working solutions in DMSO were prepared (Tab.1). The different working solutions were prepared by diluting the dye solution with the highest concentration. All solutions were filtered before use. In the wet-to-dry mode, test volumes were transferred to transparent 96 well microplates and subsequently filled with the appropriate volume of 0.1 N NaOH using a CyBi®-Well vario 96/250 µL. In wet-to-wet transfers, the microplates were primarily filled with the appropriate volume of 0.1 N NaOH using a CyBi®-Well vario 96/250 µL and then the p-NP working solution was added with tips immersed at approximately 1mm.

Test volume [µL]	Volume of 0.1 N NaOH [µL]	p-nitrophenol working solution in DMSO [mM]
5	195.0	2.4
2	198.0	6.0
1	199.0	12.0
0.5	199.5	24.0
0.2	200.0	60.0
0.1	200.0	120.0

Table 1: Experimental settings to measure the precision of the 96-channel head of the CyBi®-CrystalCreator using 25 µL tips.

After shaking (10 min, 400 rpm) and centrifugation (2 min, 2,000 rpm), the absorbance was measured with the absorption reader ELX 800 (BioTek) at 405 nm. Absorbance readings were taken at least one hour after liquid transfer.

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All pipetting methods were programmed using CyBio Control software, version 3.43 as following:

- precision tests were performed with three parallel microplates per volume and pipetting mode
- one set of 96 new tips for each volume
- piston speed: 30 rpm (characterizes the flow rate of the liquids during aspiration/dispensation; = 1.23 $\mu\text{L/s}$ with a 25 μL head)
- general stage speed: 140 rpm
- stage speed for a perfect droplet performance in the wet-to-dry pipetting mode: 20 rpm
- stage speed for a perfect dispensing performance in the wet-to-wet pipetting mode: 50 rpm
- priming of tips: at least 1x with highest tip volume
- break of 2s after every aspiration and dispensing step
- tip immersion depth 1 - 2 mm
- final volume 200 μL per well
- final dye concentration 60 μM
- all aspirations steps utilizing overstroke (optimized pipetting option)
- dispensing back of the first pipetting cycle into the reservoir
- pipetting of the target volume as part of the total volume into the test microplate
- **dry pipetting** involves dispensing the desired volume of the dye solution about 1 mm above the bottom of the dry microplate and then touching-off the droplet to the plastic ware, followed by dispensing of 0.1 N NaOH to a final volume of 200 μL ; use of the tolerance mat for the destination microplate is essential
- **wet pipetting** involves dispensing the desired volume with tips immersed approx 1 mm into the provided 0.1 N NaOH liquid
- ejecting the residual volume with maximum overstroke (blow-out) back into the source reservoir or waste
- immediate sealing of the microplates
- orbital shaking of the microplates for at least 10 minutes, subsequent incubation at room temperature for at least 30 minutes
- centrifugation of the microplates for 2 minutes at 2000 rpm
- absorption measurement performed no earlier than 30 minutes after completing the centrifugation

The results can be reproduced when these methodical details are considered.

Precision (Tab.2) is characterized by the coefficient of variation (%CV) which results from the following calculation:

coefficient of variation (%CV) = (standard deviation of parallel measurements / mean) x 100%.

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Results and Discussion

In Table 2 the obtained precision data of the 96-channel head of the CyBi®-CrystalCreator, using different volumes as well as different pipetting modes, is summarized. The precision was calculated as a percentage standard deviation (coefficient of variation = CV in %) over a 96 well microplate. Three microplates were prepared per volume and averaged.

Test Volumes p-NP in DMSO	Precision wet-to-dry [%CV]	Precision wet-to-wet [%CV]
5 µL	0.53	0.70
2 µL	0.60	0.79
1 µL	0.91	0.87
0.5 µL	1.42	1.62
0.2 µL	4.26	2.75
0.1 µL	5.93	5.24

Table 2: Overview of precision data obtained with the 96-channel head of the CyBi®-CrystalCreator for various volumes and different pipetting modes, using 25 µL tips (n=3).

The results indicate highly precise liquid handling of small volumes in both wet-to-wet and wet-to-dry pipetting modes. Down to 0.5 µL the precision data is slightly better for dry dispensing than for wet dispensing (Tab.2 and Fig.1). These very good results in the wet-to-dry pipetting mode are due to the use of a tolerance mat. For the compensation of any intolerances caused by the microplates or tips, the usage of a tolerance mat is indispensable and recommended.

A general quality of the disposables, especially the flatness of the microplate bottom and the consistency of the tip length, is essential for the outcome of wet-to-dry liquid transfers. The 96-channel head of the CyBi®-CrystalCreator allows a vertical adjustment of the stage height in steps of 1/10 mm for touching the tips to the microplate bottom in order to deposit a droplet reliably. With this height fine-adjustment capability and the tolerance mat, a high standard of precision can be achieved.

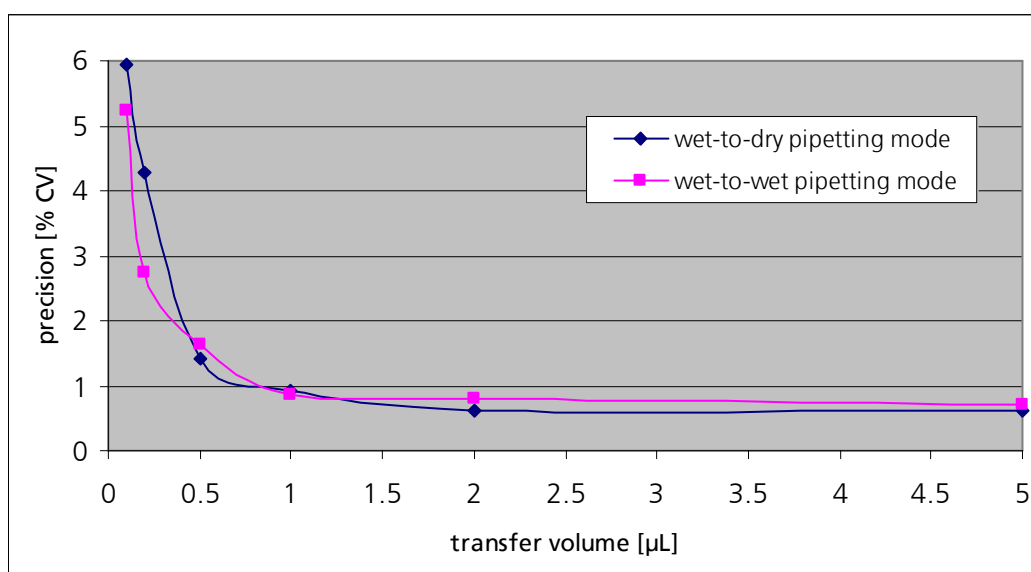


Figure 1: Precision data (absorbance measurements) obtained with the 96-channel head of the CyBi®-CrystalCreator, comparison of the pipetting modes (wet-to-dry or wet-to-wet liquid transfer) using 25 µL tips.

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The 96-channel head of the CyBi®-CrystalCreator enables pipetting down to 0.1 µL using 25 µL tips with precision errors less than 6% (Tab.2 and Fig.1) in both pipetting modes, wet-to-dry and wet-to-wet (Tab.2 and Fig.1). Because of advanced tip sealing and the high quality of the tips, continuous high precision and reproducible data over the whole microplate can be achieved (Fig.2).

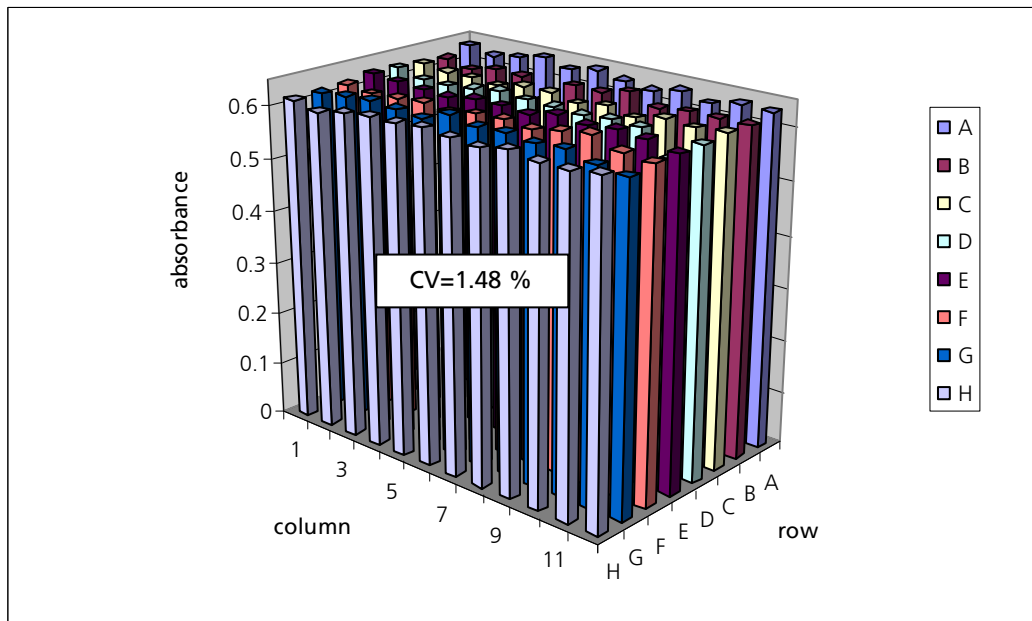


Figure 2: Precision of an example 96 well microplate: transfer of 0.5 µL (wet-to-dry mode) with 25 µL tips with an overall precision of CV=1.48 %.