

Technical Note

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Precision of the CyBi®-SELMA 96, 250 µl

Example Data for low volumes, different pipetting modes and liquids

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Introduction

CyBi®-SELMA is a semi-automatic pipettor for quick, precise and reproducible processing of 96-well and 384-well microplates. Equipped with 96 parallel working tips it is easy to operate without the need for a separate computer control system. The basic functions Pipetting, Reverse Pipetting, Dispensing and Diluting can be selected on a touch screen. For serial dilutions a special magazine with 8 tips is provided. Furthermore, flexible liquid handling parameters like volumes, pipetting heights and pipetting speeds can be defined via the touch screen. For recurrent processes all liquid handling parameters can be saved and thus recalled conveniently at any time as needed.

The precision error of the CyBi®-SELMA 96, 250 µl is specified to be CV < 2% in the volume range between 10 µl and 25 µl, and CV < 1% in the volume range between 25 µl and 250 µl. CyBio's in house specification check and quality control is performed in the wet-to-wet reverse pipetting mode with a standardized absorption method (p-Nitrophenol [p-NP] as dye dissolved in 0.1 N NaOH), which is described in detail in the CyBi®-SELMA manual. In this study this absorption method was used to generate example precision data for different pipetting modes, using dye in 0.1N NaOH or DMSO. The data especially cover the volume range below the specification limit to indicate the threshold volumes at the different experimental conditions.

The aim of this study is to summarize all our liquid handling experience with the CyBi®-SELMA 96, 250 µl.

The data may support the choice of the right pipetting mode and pipetting parameters for a broad range of customer specific applications and show the volume limits which vary with pipetting conditions and liquids.



Figure 1: The CyBi®-SELMA 96, 250 µl with deep well tips and 2 working positions

Materials

Devices

- » CyBi®-SELMA 96, 250 µl (CyBio AG, # OL 7001-26-200)
- » ELx800 Absorption Reader (BioTek Instruments Inc.)
- » Centrifuge Sigma 6K15 with Microplate Rotor N° 11150 (SIGMA Laboratory Centrifuges)
- » IKA Microplate Shaker MTS4 (IKA GmbH & Co.KG)

Reagents

- » p-Nitrophenol (p-NP, Sigma # 104-8)
- » DMSO (Merck, Darmstadt, # 1.2931.1000)
- » NaOH (Roth, Karlsruhe # 6771.3)

Consumables

- » Tip Tray 96 -250 µl deep well (CyBio AG, # OL 3800-25-559-N)
- » OmniTrays (Nunc # 140156) as disposable reservoirs
- » Greiner PS transparent flat bottom microplates 96 (Greiner bio-one # 655 101)
- » Adhesive foil (Nunc # 236269)

Methods

The precision test was performed using transparent 96 well microplates with a final volume of 200 µl 0.1 N NaOH per well and a final dye concentration of 120 µM. The adequate concentration of the p-NP working solution for the different test volumes is shown in Table 1.

Table 1: Volumes of p-NP dye and 0.1 N NaOH to measure the precision of the CyBi®-SELMA 96, 250 µl

Test volume [µl]	p-NP [mM]	0.1 N NaOH [µl]
10	2.4	190
5	4.8	195
2	12.0	198

The different p-NP working solutions were prepared by diluting the dye solution with the highest concentration. All solutions were filtered and used at room temperature.

In the wet-to-dry mode, the test volumes were transferred to empty transparent 96 well plates with tips only tenth of mm above the plate bottom and then the plates were filled up with the appropriate volume of 0.1 N NaOH.

In the wet-to-wet mode, first the microplates were filled with the appropriate volume of 0.1 N NaOH and then the p-NP working solution was added with tips immersed at about 1 mm.

In the mode "Dispensing" the first liquid transfer was dispensed back into the reservoir and the 3 following transfers were analyzed.

In the mode "Diluting" 0.1 N NaOH was aspirated followed by an air gap and the test volume of dye solution. All liquid was dispensed together into the microplate.

For all tests tips were primed 3 x with 250 µl dye solution or in the mode "Diluting" with 0.1 N NaOH, respectively. The piston speed was adjusted to 40 µl/s (slow) and the stage speed to 30 mm/s.

The microplates were sealed immediately, were shaken for 10 min at 400 rpm and then they were centrifuged for 2 min at 2,000 rpm. The absorbance readouts at 405 nm were taken at least one hour after the final liquid transfer.

For reproduction of the results these experimental conditions have to be considered in all details.

Precision was calculated as percentaged standard deviation (coefficient of variation = CV in %) for each microplate. Three microplates were prepared per test volume, liquid and pipetting mode and the results were averaged.

Results and Discussion

In Table 2 the CyBi®-SELMA 96, 250 µl precision data for the different test volumes, liquids and pipetting modes are summarized.

The results indicate that the CyBi®-SELMA 96, 250 µl allows highly precise liquid handling at 10 µL and below in all pipetting modes. The CV-value for reverse pipetting of 10 µl p-NP in NaOH in the wet-to-wet mode (CV=0.6%) is well within the specified value of CV < 2%. It can be assumed, that for volumes higher than 10 µl the precision is comparable with the 10 µl data or even better.

For both p-NP in DMSO and 0.1 NaOH with decreasing volumes the results for wet-to-wet transfer were slightly better compared to wet-to-dry transfer.

Table 2: Precision data (n=3) obtained with the CyBi®-SELMA 96, 250 µl for various test volumes, test liquids and pipetting modes **with 3x priming of tips**. Volumes resulting in CV values >10 % are not recommended (n. r.)

Mode	Volume dye [µl]	p-NP in DMSO wet [% CV]	p-NP in DMSO dry [% CV]	p-NP in NaOH wet [% CV]	p-NP in NaOH dry [% CV]
<u>Pipetting</u>					
	10	1.1	1.0	0.6	1.0
	5	1.8	2.1	0.8	2.4
	2	2.3	5.4	5.8	7.9
<u>Reverse Pipetting</u>					
	10	1.0	1.0	0.6	1.2
	5	0.9	2.0	0.9	2.5
	2	1.9	3.6	2.5	n. r.
<u>Dispensing</u>					
	10	1.0	1.5	0.7	2.4
	5	1.1	2.4	1.2	3.1
	2	1.7	2.2	2.9	n. r.
<u>Diluting</u>					
	10	0.9	-	1.0	-
	5	1.1	-	2.0	-
	2	1.6	-	n. r.	-

In most cases the precision data obtained with p-NP in DMSO are a bit better compared to that with p-NP in NaOH because of the lower surface tension of the DMSO test solution. The complex interaction between the physiochemical characteristics of the tip surface and the properties of the liquids determine the threshold volumes for the different pipetting modes. They are of increasing importance for decreasing volumes.

In some applications, especially in the field of molecular biology, the amount of sample is limited and thus does not allow a multiple priming of the tips with the complete tip volume immediately before starting the actual liquid transfer. The insufficient air equilibration inside of the tips in combination with high surface tension of the test liquid and the short piston movement behind this

low volume handling may result in difficulties to dispense volumes precisely below the specification limit, e.g. 5µl or lower. In such cases the liquid transfer was performed in the mode "Diluting", starting with the aspiration of a small air volume instead of the diluent. In the final aspiration step, the test solution was aspirated and then dispensed together with the additional air volume into the destination plate. In this way the marginal movement of the pistons to aspirate and dispense the low destination volume precisely was optimized. This transfer was finished with a mixing routine. Example data for this specific liquid transfer mode to handle volumes below the specification limit without priming are shown in Table 3. In this way, at our experimental conditions volumes as low as 3 µl could be transferred with the CyBi®-SELMA 96, 250 µl reliably with precision errors less than 5 %.

Table3: Precision data of low test volumes obtained with the CyBi®-SELMA 96, 250 µl **without priming of tips** in the "Diluting" mode (aspiration of 15 µl air instead of diluent, air gap, aspiration of the test volume, dispensing into the prefilled destination plate, 3 mixing cycles with 150 µl, n=3)

Mode	Test volume [µl]	p-NP in NaOH wet [%CV]
Diluting	5	1.1
(with air instead of diluent)	4	2.1
	3	4.3
	2	9.5

The precision results of this study meet the specifications for the CyBi®-SELMA 96, 250 µL and agree with precision data, which were measured on the CyBi®-Well vario 96/250 µl Head with comparable pipetting parameters (1). The CyBi®-Well 96/250 as well as the CyBi®-Well vario with the 96/250 µl Head and the CyBi®-SELMA 96, 250 µl are working comparably based on CyBio's precise and reliable pipetting technology.

Generally a CyBi®-SELMA 96, 25 µl is the best choice for the precise handling of volumes lower than 10 µl. However, on a limited scale it's possible to use the CyBi®-SELMA 96, 250 µl also below this specification cutoff volume if care is taken for optimized pipetting approaches.

References

(1) Katrin Undisz, Pruefer H. and Hermann H.: Precision of the CyBi-Well vario 96/250 µl Head, Example Data of different Liquids, Pipetting Modes and Disposable Tips with Fluorescence Readout; Technical Note, <http://www.cybio-ag.com>

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