

CyBio

CyBi[®]-Drop

The Care Program for Cells

100% Cells/100% Zellen/
100% Celler/100% Cellule/
100% Клетки/100% Cellen/
100% Células/100% 細胞



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CyBi®-Drop – General Features

Over the last few years, the importance of cell-based assays has increased considerably. And this has seen the trend move away from biochemical assays and move increasingly towards more physiologically relevant assays that deliver more functional information. Consequently, cell-based assays are finding more and more applications and proving to be increasingly important in the Primary and Secondary Screening, as well as the ADME/Tox Screening. A further significant trend that is occurring lies in high content screening, which allows the execution of different assays at the same time and at the same well.

All automations have to adapt to the new requirements as the handling of cells is different to the biochemical assays that already exist. On this account, there are new problems which have to be solved. Examples of such problems include the homogenous distribution of cells in each well, the vitality of the cells after dispensing and the preparation of a cell suspension.

CyBio, the pioneer in automated liquid handling is therefore able to apply its considerable knowledge to deliver a workstation for gentle and sensitive cell handling.

With the new CyBi®-Drop, CyBio represents a contact-free dispenser with specially developed attributes, one such features being the unique cell recirculation of the dispensing combs. Following cell dispensing, the CyBi®^v-Drop achieves homogeneous cell distribution, ensuring excellent cell viability and minimal cell stress. The CyBi®-Drop provides a high variety of dispensing combs to enable different types of liquids – such as assay reagents, proteins, buffers and organic solvents – to be dispensed with the highest levels of both precision and speed.

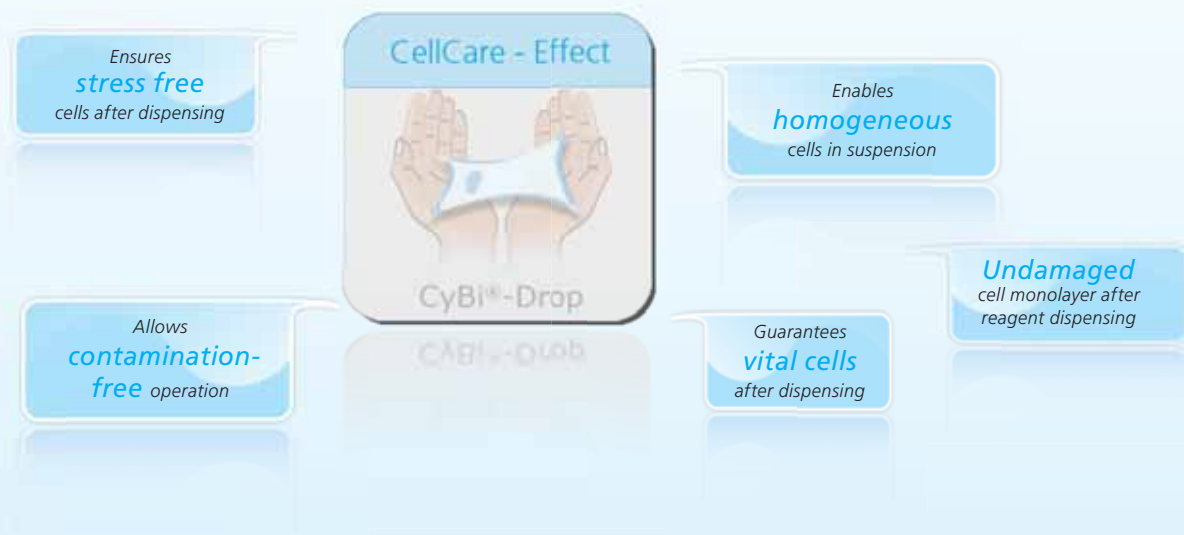


The CellCare – Effect

Outstanding cell dispensing

The CyBi®-Drop provides a range of impressive features designed to protect your cells during the dispensing process. For example, specially developed cell dispensing combs with a cell compatible material avoid cell-settling effects and clogging of the nozzels. Furthermore, the adaptation of the diameter of the nozzels enables the perfect dispensing of cells if cells form aggregates and the increased diameter reduces the mechanical stress for cells. An additional important and beneficial function is the possibility of circulating the cells before dispensing. This function enables the recirculation of the cell suspension and provides for the same amount of cells per shoot as well as an equal distribution of cells at any time.

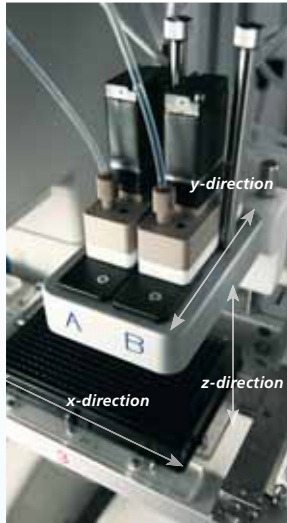
All of these features combined together mean that the CyBi®-Drop is the ideal cell dispenser and ensures gentle cell dispensing. As a result, you will experience vital and stress free cells, homogeneous suspension in each well after the dispensing process and assays that are always reproducible.



Flexible and easy adjustment of the dispensing parameter

The CyBi®-Drop enables you to adjust all of the necessary dispensing parameters, such as pump speed, volume per shot and number of shots. This option in turn guarantees gentle cell dispensing on adherent cell monolayers without any disruption. Furthermore, the fact that you are able to adjust the characteristic curve delivers many advantages and ensures the perfect set-up for each dispensing liquid which will in turn enable you to derive the best results.

The Right CyBi®-Drop Caters for All Requirements



3D Technology

The CyBi®-Drop is very modular and can be expanded at any time. It also provides many different possibilities to upgrade the device to create a higher throughput. In particular, the CyBi®-Drop 3D function is a high-precision y-drive and z-drive unit that allows for the extremely accurate carrying of the comb holder unit together with the dispensing comb – critical requirements for the perfect use of 1536-well microplates.

The CyBi®-Drop: Truly user friendly

The CyBi®-Drop can be equipped with several pumps and dispensing combs at the same time, and either two or four pumps are available. This means that up to four different liquids can be used at the same time on one microplate. Therefore annoying processes such as emptying, cleaning the tubes and filling the tubes with new liquids can now be avoided.

In addition, contamination is not an issue. The sublet and all-purpose comb holder provide for an easy exchange of the dispensing combs, allowing for the use of the optimal dispensing comb for each application. A further advantage lies in the volume control that is enabled via software. This allows for different volumes to be applied safely and reproducibly adjust within one program. The CyBio® Composer is user friendly, flexible and means you can start the CyBi®-Drop in less steps.

Configuration possibilities

The CyBi®-Drop and the CyBi®-Drop 3D enables gentle and perfect dispensing of a high variety of liquids and cells in a wide range of microplate formats. Through the following matrix, you should be able to ascertain the right dispenser configuration for your tasks.

		CyBi®-Drop	CyBi®-Drop 3D
Usable microplate formats	96	X	X
	384	X (only with 16-channel comb)	X
	1536	-	X
	SW	X	X
	DW	-	X
Dispensing volume range	0.5 µL – 2 µL	X (only with 8-channel comb)	X (only with 8-channel comb)
	2 µL – 8 µL	X	X
	8 µL – 15 µL	X (only with 8-channel comb)	X (only with 8-channel comb)
Number of dispensing reagents	2	X	X
	4	X	X
Kind of dispensing reagents	Cells	X	X
	Other liquids	X	X
Kind of equipment	Stand alone	X	X
	With stacker	X	X
	Add on in an integration	X	X

The CyBi®-Drop: Truly Unique

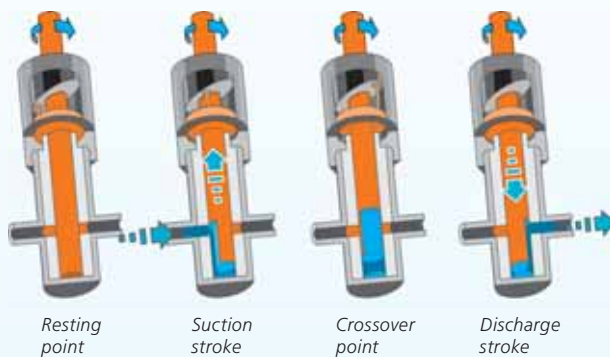
Experienced technology

Most of dispenser require peristaltic pumps and valves to control dispensing. However, flexible tubing components and lack of positive displacement adversely affects the durability of the pump and the consistency of the dispensing. Furthermore, the use of valves causes a degradation of the performance and results of the valves and the system.

Advanced pump technology

The fundamental basis of the CyBi®-Drop is a ceramic pump that has been developed to eliminate valves and time consuming maintenance. Cavities work instead valves in a rotating piston. While the cavity is turned to the inlet port, the piston starts the intake stroke and the fluid pull into the cylinder. In addition, the rotation opens the cavity to the outlet port and the piston starts the displacement stroke.

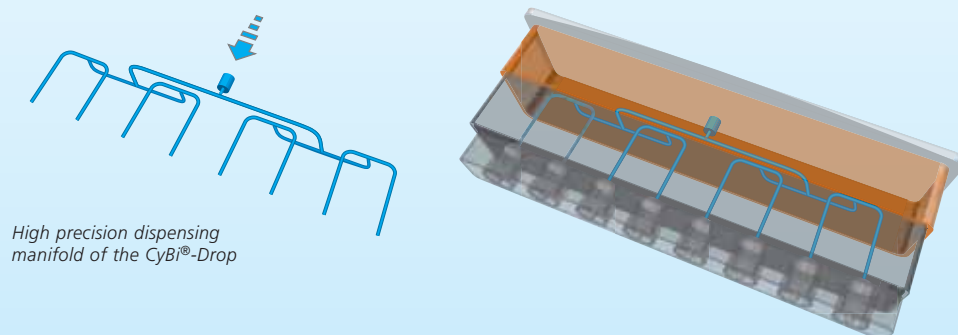
This enables fluid to leave the cylinder and fill the awaiting well. The CyBi®-Drop user can define the dispensing volume via software.



Ceramic pump operation with user-defined variable stroke

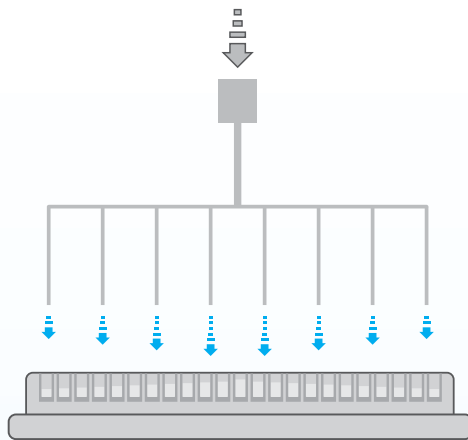
Evenly distribution for an ideal flow

The CyBi®-Drop manifold uses a two step division system milled directly into the nozzle. This ensures a perfectly uniform distribution and compared to other fluid distribution schemes. Furthermore, the manifold is positioned directly above the nozzle outlet to eliminate flow-dampening effects of joints, diameter changes or long tubing.

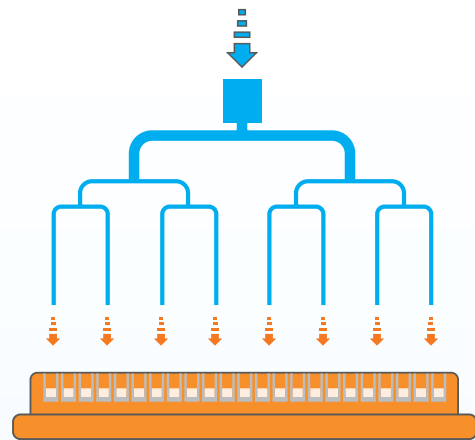


High precision dispensing manifold of the CyBi®-Drop

CyBio's patented dispensing comb with 8 or 16 channels. Patent no. EP 1 036 594 B1



Uneven dispensing across microplate caused by unsuitable flow distributor.



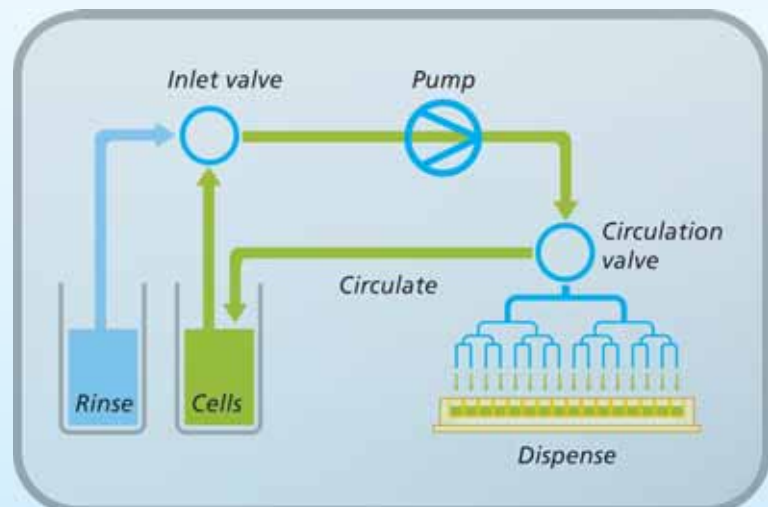
Maximum dispensing accuracy and reproducibility with CyBio's patented two step division system.

Essential for the setup of cell-based assays

More and more cell-based assays are carried out in the majority of screening labs due to the important results created with living cells. In the process of the assay setup, cell seeding is a crucial step with a major impact on the data quality. This makes the cell dispenser a key component for cell-based screening.

A unique cell recirculation function

That is available for the CyBi®-Drop non-contact dispenser only, eliminates cell settling effects and enables homogeneous distribution of cells while they are being dispensed. This results in a level of uniformity in cell dispensing, which could not be achieved so far. Dedicated cell dispensing combs with cell-optimized fluid paths make the CyBi®-Drop the ideal cell dispenser, featuring excellent cell viability and minimal cell stress. Therefore the physiology and pharmacology of the cells is retained and maximal relevance of the results is provided.



Schematic diagram of the unique cell recirculation function

Experience Optimal Cell Dispensing

The following figures show selected experimental data to characterize the performance of the CyBi®-Drop / CyBi®-Drop 3D from different application points of view.

Precise dispensing of reagents

The specification of the precision error for the different dispensing combs and different volume ranges are summarized on page 10. The example precision data in Fig. 1 and Fig. 2 show that even better results can be expected in typical reagent dispensing applications. For further experimental details, please see (1).

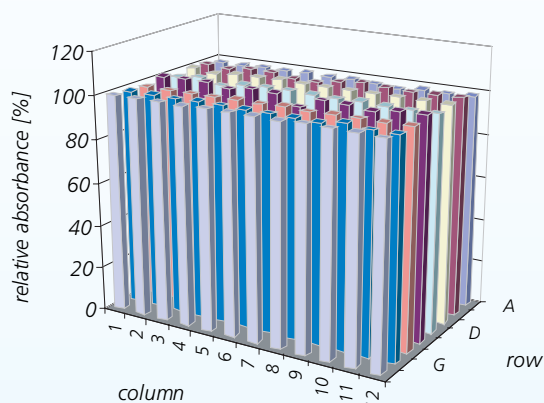


Fig. 1: Example precision of dispensing 2 μL 6 mM p-Nitrophenol solution in 0.1N NaOH into 100 μL 0.1N NaOH per well in a 96-well microplate with an 8-channel dispensing comb and steel nozzles, pump speed = 400 rpm, mean value = 100 %, **CV = 1.02 %**.

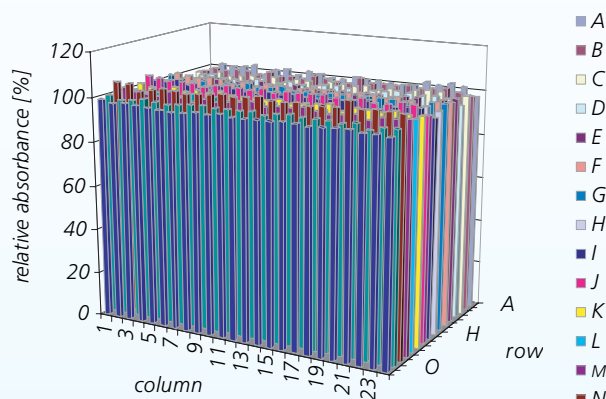


Fig. 2: Example precision of dispensing 2 μL 3 mM p-Nitrophenol solution in DMSO into 50 μL 0.1N NaOH per well in a 384-well microplate with a 16-channel dispensing comb and peek nozzles, pump speed = 600 rpm, mean value = 100 %, **CV = 1.8 %**.

Gentle dispensing of reagents on adherent cell monolayers

In cellular assays, the addition of assay reagents or media into the wells of an assay plate with an adherent cell monolayer on the well bottom is often required. And to ensure the correct evaluation of the assay results, the complete preservation of the cell monolayer is an essential precondition.

The flexible adjustment of the pump speed and the freely selectable pump stroke numbers per well (also for smaller volumes) provide for the successful optimization of the dispensing parameters for such typical cellular applications.

In Fig. 3, the stereomicroscopic picture of the disruption of a CHO cell monolayer after dispensing 10 μL cell medium with one stroke and high pump speed is shown.

Meanwhile, Fig. 4 shows the complete preservation of the CHO cell monolayer following the dispensing of 5 times 2 μL medium with a reduced pump speed. For further experimental details, please see (2).

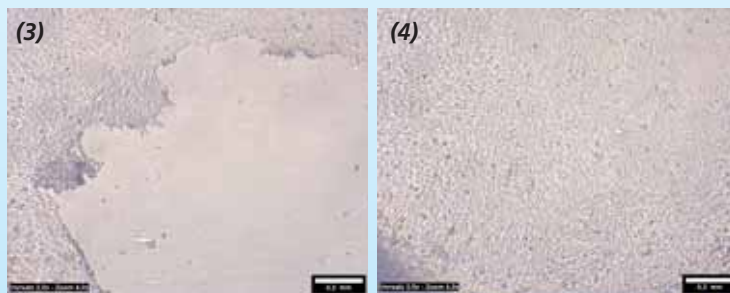


Fig. 3: Disruption of the CHO cell monolayer after the dispensing of 10 μL medium in a 96-well microplate with 100 μL medium / well (8-channel dispensing comb with steel nozzles, pump speed 400 rpm, stereo microscope ASKANIA MZM 1).

Fig. 4: Complete preservation of the CHO cell monolayer after the dispensing 5 x 2 μL medium in a 96-well microplate with 100 μL medium / well (8-channel dispensing comb with steel nozzles, pump speed 300 rpm, stereo microscope ASKANIA MZM 1).

Homogeneous distribution of cell suspensions into microplates

In cellular screening applications, the homogeneous distribution of cell suspensions into microplates is a challenging task. This is because of the high probability of cell sedimentation effects. The automated circulation function of the CyBi®-Drop combs allows for the real time priming of the tube system with homogeneous cell suspension immediately prior to the dispensing starting. Fig. 5 (A) shows the result of dispensing a CHO cell suspension without recirculation in a 96 well microplate. In columns 9-12, the cell sedimentation effects are clearly observable and the precision of the resulting cell viability signal over the whole plate without cell recirculation is **CV = 7.6 %**. Meanwhile, Fig. 5 (B) shows a comparable experiment with cell recirculation. The precision of the resulting cell viability signal over the whole plate is much improved (**CV = 3.4 %**) with cell recirculation.

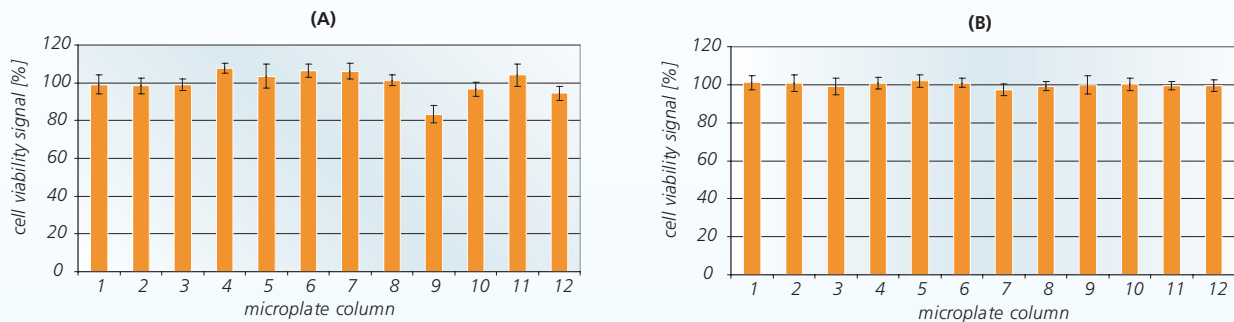


Fig. 5: Dispensing of $10 \times 5 \mu\text{L}$ CHO cell suspension (2×10^6 cells/mL, 8-channel dispensing comb with peek nozzles) without (A) and with cell recirculation (B) into $50 \mu\text{L}$ cell medium per well and analyzing the cell viability with the CellTiter-Blue® assay, bars indicate the mean cell viability signal of each column compared to the mean value of the whole microplate (=100%).

Preservation of suspension cells and their functionality even after multiple cell recirculation cycles

It is recommended to use the recirculation function for every new plate to ensure the homogeneous distribution of cell suspensions in cellular screening applications. The influence of multiple cell recirculation cycles on the cell stability and functionality was examined microscopically and using a CASY® Cell Counter and Analyzer System. For experimental details, please see (3). Fig. 6 shows a microscopic picture of K-562 blood cells following ten recirculation cycles and dispensing with the CyBi®-Drop 3D. No differences were observed microscopically compared to the original cell suspension.



Fig 6: K-562 cell suspension following 10 recirculation cycles (pump speed 20 rpm, 2.5 mL per cycle, LSM 510 META), cells we maintained, mean cell diameter = 16 μm .

After 1 and 10 recirculation cycles, cell populations were analyzed in a CASY® Cell Counter and Analyzer System to compare the particle size distribution. As shown in Fig. 7, the particle size profile following 10 times cell recirculation and dispensing with the CyBi®-Drop 3D is very similar compared to the original cell suspension. This indicates the preservation of the cells and their functionality even after multiple cell recirculation cycles. In addition, the metabolic capacity of the cells remained stable (CellTiter-Blue® Cell Viability Assay (Promega)). For further data and experimental details, please see (3).

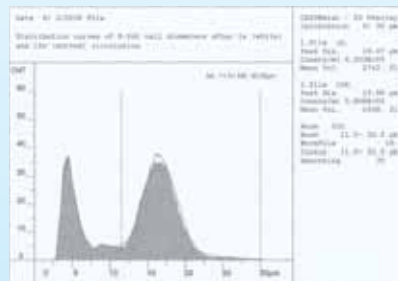


Fig. 7: CASY® 2D Overlay of the K-562 blood cell suspension following 1 and 10 recirculation cycles and dispensing with the CyBi®-Drop 3D.

References: 1. Undisz, K. et. al. (2008); "Precision of the CyBi®-Drop 3D, Example Data with Different Dispensing Combs and Liquids", TechNote CyBio AG, www.cybio-ag.com
 2. Undisz, K. et. al. (2008); "Handling of Adherent Cells with the CyBi®-Drop 3D, Example Data", Application Note CyBio AG, www.cybio-ag.com
 3. Undisz, K. et. al. (2008); "Handling of Cell Suspensions with the CyBi®-Drop 3D, Cell Viability Studies", Common Application Note with the Department Infection Biology of the Leibniz Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, www.cybio-ag.com

Technical Data

General specifications

	CyBi®-Drop	CyBi®-Drop 3D
<i>Liquid handling principle</i>	Rotation piston pump + patented multichannel droplet generator	
<i>Number of dispensing combs</i>	2 or 4	
<i>Usable dispensing combs</i>	8-channel standard dispensing comb, 250 µm nozzels, stainless steel 8-channel standard dispensing comb, 380 µm nozzels, PEEK 8-channel cell dispensing comb, with cell recirculation, 380 µm nozzels, PEEK 16-channel standard dispensing comb, 250 µm nozzels, stainless steel 16-channel cell dispensing comb, with cell recirculation, 380 µm nozzels, PEEK	
<i>Plate formats</i>	96, SW or DW* 384, SW or DW* (only with 16-channel dispensing comb)	96, 384, 1536 SW & DW* (with automated height adjustment)
<i>Dispense channels</i>	8 and 16	
<i>Dispensing increments</i>	0.1 µL	
<i>Dead volume</i>	< 4 mL, reusable	

* SW-Shallow Well; DW-Deep Well

CyBi®-Drop – dispensing comb specifications

	No. of dispense channels	Volume range	Precision*	Dispensing speed per plate (incl. 5 waste shots)
<i>8-channel dispensing comb, stainless steel</i>	8	0.5 – 15 µL/ per shot	0.5 µL CV < 10% 1 µL CV < 5% 5 µL CV < 3%	96: 1 shot / well in 10 sec 10 shots / well in 25 sec 25 shots / well in 45 sec
<i>8-channel dispensing comb, PEEK</i>	8	1 – 15 µL/ per shot	1 µL < CV 10% 5 µL < CV 3%	384 (only with CyBi®-Drop 3D) 1 shot / well in 35 sec 5 shots / well in 60 sec
<i>Cell dispensing comb 8-channel, PEEK</i>	8	1 – 15 µL/ per shot	1 µL < CV 10% 5 µL < CV 3%	1536 (only with CyBi®-Drop 3D) 1 shots / well in 85 sec
<i>16-channel dispensing comb, stainless steel</i>	16	1 – 8 µL/ per shot	1 µL < CV 10% 5 µL < CV 4%	384: 1 shot / well in 20 sec 10 shots / well in 45sec
<i>Cell dispensing comb 16-channel, PEEK</i>	16	2 – 8 µL/ per shot	2 µL < CV 10% 5 µL < CV 4%	1536 (only with CyBi®-Drop 3D): 1 shot / well in 45 sec

*standard absorbance protocol

Expandable Each and Every Time

The CyBi®-Drop provides five different dispensing combs depending on application and throughput. The use of the sophisticated 16-channel dispensing comb represents an excellent benefit as it increases throughput. Furthermore, the combination with stackers and products of the CyBi®-Well family result in not only a considerable increase of the throughput, but also a wide variety of applications.

In terms of lab space, the CyBi®-Drop makes a great impression through its narrow footprint and it can use the linear microplate carriage of CyBio's multichannel pipettors. This means it efficiently integrates with the CyBi®-Well and CyBi®-Well vario. The microplate carriage moves the plates under the comb and carries them off again following filling. The microplate is positioned precisely column by column under the dispensing comb of the CyBi®-Drop. This is to ensure the efficient and accurate filling in continuous mode for single dispensing and in step mode for multiple dispensing. For stand-alone use and for integration into robotic systems, the CyBi®-Drop uses a microplate transport system where lengths can be chosen according to the automation needs. Equipped with up to 4 stackers, CyBi®-Drop can process up to 420 plates per run without the need for user intervention.

The following examples demonstrate the combination possibilities and application areas for the CyBi®-Drop:

CyBi®-Drop with stacker

for high throughput plate filling



CyBi®-Drop with multichannel pipettors (CyBi®-Well/ vario and also with the new CyBi®-WellFlex/ vario)

for complex assays in the field of ADME/Tox,
cell-based assays and more

CyBi®-Drop in integration

for automation of whole high throughput
screening campaigns



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