

Application Note

CyBi[®]-RoboSpense – Performance Characterization and Demonstration of Assay Optimization Capabilities

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1. INTRODUCTION

The CyBi[®]-RoboSpense is a liquid handling workstation equipped with up to 8 individually addressable liquid channels. The system either operates with washable fixed steel probes or with adapters for disposable tips. The tip adapters enable the use of 4 different tip sizes: 1100µl, 300µl, 50µl and 10µl. Therefore, the CyBi[®]-RoboSpense supports applications in a large dynamic volume range without user interaction (1 to 1000µl at least). Since needles or tips allow equidistant spreading CyBi[®]-RoboSpense is capable of flexible tube-to-plate pipetting. Integrating a robotic microplate gripper on the platform is an option to allow easy plate transfer within deck positions and to adjacent liquid handling and reading devices. Thus, the instrument adds a new dimension of flexibility to the CyBio instruments portfolio.



Fig. 1: CyBi[®]-RoboSpense, flexible liquid handling workstation

This application note evaluates key performance parameters of the CyBi[®]-RoboSpense liquid handling platform and the applicability of the instrument for assay optimization processes. Precision and linearity were tested by absorbance measurements while accuracy was determined by gravimetric methods. As an example for the use of the CyBi[®]-RoboSpense in biological assay

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optimization the enzymatic lactate dehydrogenase (LDH) assay was chosen. Concentrations of LDH, NADH (nicotinamide adenine dinucleotide) and pyruvate were varied within the same plate for assay optimization to demonstrate the instrument's accurate liquid handling and flexibility.

2. MATERIALS AND METHODS

Instrument:	CyBi®-RoboSpense, disposable tips, 8 channels, gripper, hotel
Microplates:	Greiner, PS microplate 96 well, #655101
Reader:	Bio-Tek Elx800 at 405 nm, BMG Polarstar at 340 nm
Buffer:	Sodium phosphate, Sigma #S-9390, 10 and 100 mM at pH 7.5, containing 0.01% TX-100, Sigma #T-9284
Dye:	p-Nitro-Phenole, Sigma #104-8, dissolved in 10 mM buffer
NADH:	Nicotinamide Adenine Dinucleotide, red. form, Sigma #N-6005
LDH:	Lactate Dehydrogenase, Sigma #L-2625
Pyruvate:	Sodium Pyruvate, Sigma #P2256

Absorbance measurements for dye testing:

- Buffer (10 mM) was filled into the wells of the plates
- Dye was added in the desired concentration
- Centrifugation for 2 min at 700g
- Microplates were lidded and incubated for 30 min in a humidified chamber
- Absorbance measurements were performed at 405 nm, five flashes

LDH assay:

- LDH activity was determined by measuring the decrease of NADH concentration via a decreasing absorbance signal. The decrease of NADH concentration correlates to the reduction of pyruvate by LDH.
- The three assay reagents, each solubilized in 100 mM buffer, were pipetted into the wells of a microplate. Concentrations of LDH, NADH and pyruvate were varied on a single microplate according to the patterns shown in Fig 2.
- For volume normalization 100 mM buffer was added to all wells to obtain a final volume of 180 µl.
- The order of dispensing was buffer, LDH, NADH and pyruvate.
- After pipetting and subsequent centrifugation (2 min, 700 g) absorbance measurements (340 nm, five flashes) of the plate were performed at time intervals of 15 minutes.
- The plate was kept lidded between measurements in a humidified chamber.

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Layer 1 LDH											
60 μ L											
1.67 U/mL LDH											
		40 μ L									
		1.36 U/mL LDH									
				20 μ L							
				0.68 U/mL LDH							
								no LDH			
Layer 2 NADH											
60 μ L											
0.45 mM NADH											
		40 μ L									
		0.3 mM NADH									
				20 μ L							
				0.15 mM NADH							
Layer 3 pyruvate											
60 μ L											
4 mM pyruvate											
		30 μ L									
		2 mM pyruvate									

Fig 2: Patterns, dispensed volumes and end concentrations of the LDH assay. Only the layers of the bio-reagents addition are shown. Not shown is the layer for the compensation buffer.

3. RESULTS

3.1 PERFORMANCE CHARACTERIZATION

Fig. 3 demonstrates absorbance raw data for a 100 μ l dispense resulting in variation coefficients between 0,6 % CV and 1,1 % CV for single channel pipetting and 0,9 % CV calculating across the whole 96-well plate. Fig. 4 shows results of linearity testing demonstrating excellent correlation ($r^2 = 0.9999$).

Table 1 summarizes the results on precision and accuracy of the CyBi®-RoboSpense for the different tip sizes tested. Variation coefficients are based on all 96 wells of the respective plate.

Volume (μ l)	Precision % CV	Accuracy %	Tip Type
1	< 8%	not determined	50 μ l tips
5	< 2 %	< 4%	50 μ l tips
10	< 1,5 %	not determined	50 μ l tips
100	< 1%	not determined	300 or 1000 μ l tips

Table 1: Precision and accuracy data

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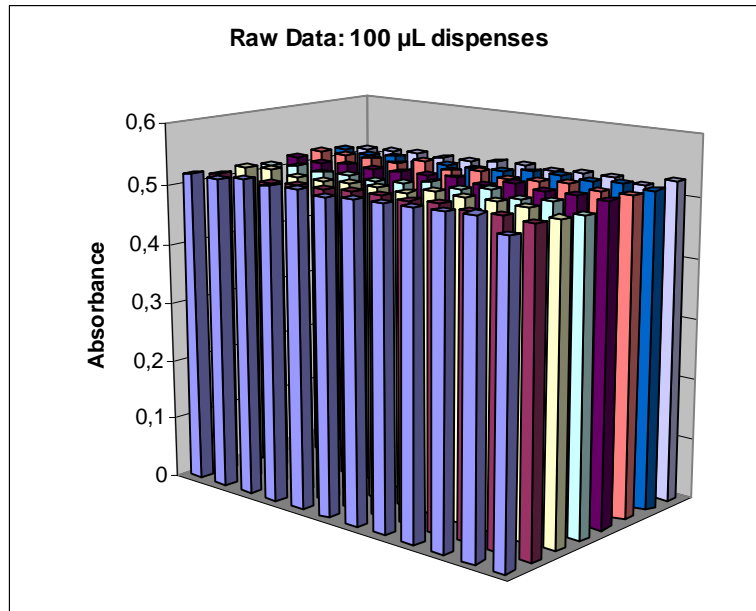


Fig 3: Raw data of a 100 µl pipetting experiment

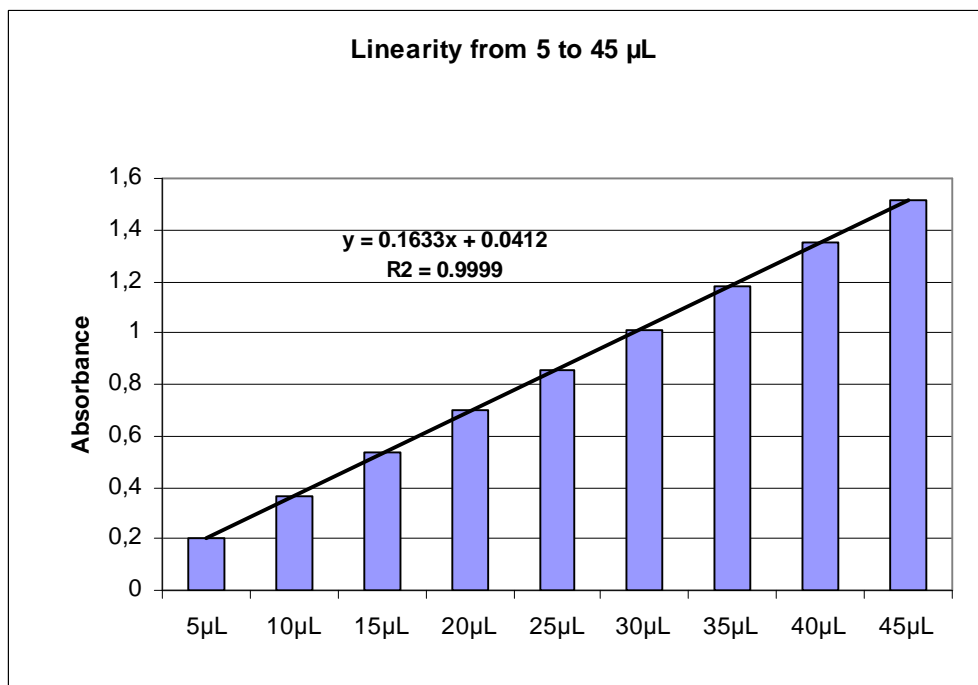


Fig 4: Determination of pipetting linearity of the instrument

The data obtained demonstrate that the CyBi®-RoboSpense liquid handling workstation fulfils all precision, linearity and accuracy standards of modern liquid handling as demanded for example in ADME-testing and assay development.

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3.2 LDH ASSAY OPTIMIZATION

Optimization of the LDH assay affords dispensing of complex reagent patterns into microplates. The flexibility of the CyBi[®]-RoboSpense hardware and software suits such tasks perfectly. The software of the CyBi[®]-RoboSpense supports easy and fast programming. Automated filling routines are a key feature to make programming of complex plate patterns simple. As an example, programming the LDH assay optimization plate as described in Fig. 2 took less than 15 minutes. In addition, CyBi[®]-RoboSpense shows fast movements of the pipetting devices. Execution of the whole pipetting procedure was performed in less than 10 minutes. The final dispense starting the assay reaction was completed in less than 2 minutes. This example demonstrates that CyBi[®]-RoboSpense enables automation of even time critical enzymatic assays.

Fig 5 shows a typical readout of an assay optimization microplate filled according to the patterns described in Fig. 2. Here the data after 15 min incubation are demonstrated. The absorbance readings of the quadruplicates show a good correlation for each set of reagent concentrations demonstrating the accurate performance of the CyBi[®]-RoboSpense.

The following additional conclusions can be drawn from the readout:

The different LDH (and pyruvate) concentration levels clearly can be distinguished when higher concentration levels of NADH (0.45 and 0.3 mM) are involved. This indicates that the assay is sensitive to variations in LDH concentration if a NADH concentration higher 0.3mM is applied. At lower NADH levels the assay does not show any sensitivity to LDH concentrations less than 0.68 U/ml.

The data also allow a rough estimation of a screening window by calculating Z-prime factors (see Zhang/Oldenburger, JBS 1999) from the different settings. Taking into account the data from wells with highest LDH level and from wells without any enzyme Z-prime factors of larger than 0.6 result for the 0.45 mM NADH level. This indicates a good screening window. However, since the number of samples per parameter is too small additional experiments are needed to verify results.

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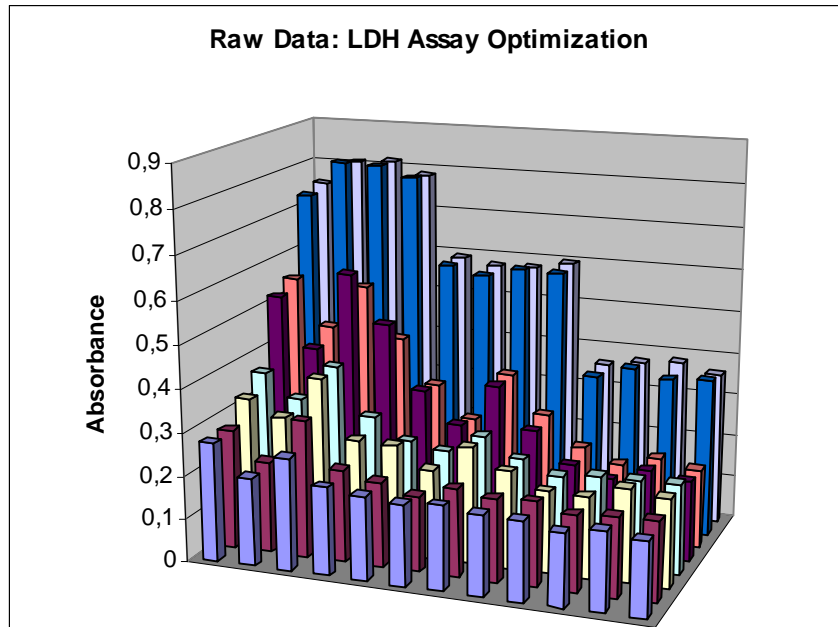


Fig 5: Raw data of an LDH optimization experiment.

Fig 6 shows the kinetics of the LDH assay. Conversion of the enzyme in relation to incubation time is demonstrated for different pairs of NADH/pyruvate levels keeping the LDH concentration constant (0.68 U/ml). The graphs allow the conclusion that for further assay optimization an increase in incubation time to at least 30 minutes will maximize the screening window. Kinetics for each concentration level show high similarity. This indicates that the CyBi® - RoboSpense features a large dynamic volume range and therefore is ideally suited for the automation of biological assays.

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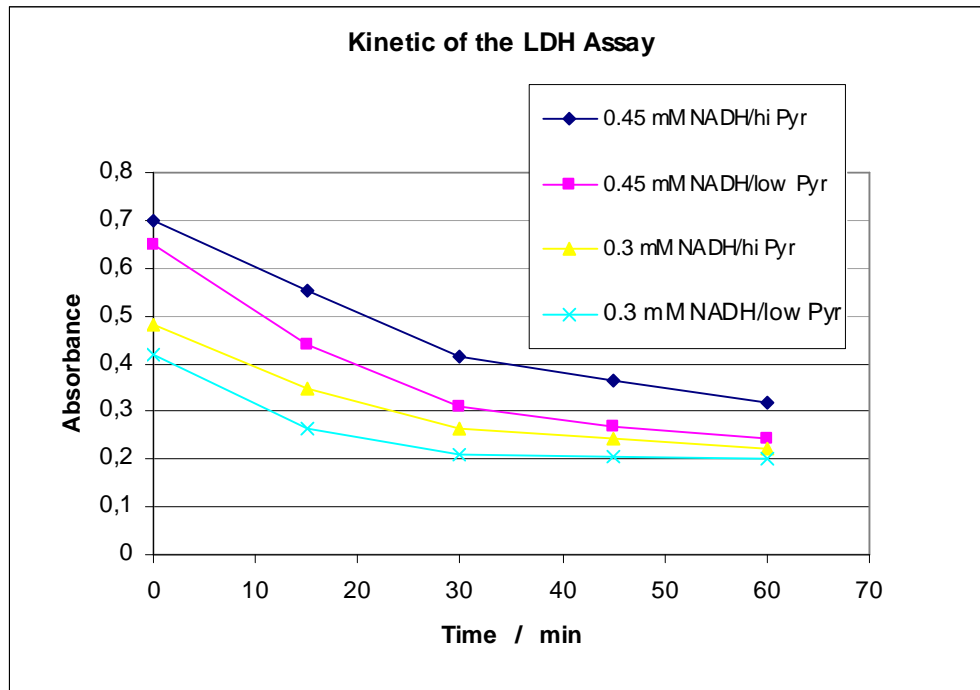


Fig 6: Kinetics of the LDH assay for different NADH/pyruvate combinations at a LDH level of 0.68 U/ml.

4. SUMMARY

The liquid handling performance of the CyBi[®]-RoboSpense was tested for different volumes and disposable tip sizes. 1100µl, 300µl and 50µl tips were used for the experiments. It was demonstrated that pipetting precision meets highest standards resulting in CVs as outlined in Table 1. Low volume pipetting will be evaluated in more depth through use of 10µl tips in the near future. First accuracy measurements resulted in values better than 4% at 5 µl volume. Evaluation of the CyBi[®]-RoboSpense for LDH assay optimization demonstrated the advantages of the instrument's flexibility in both, hardware and software features. The powerful software proved its usability for fast and easy programming of complex pipetting routines. The operating speed of the system turned out to be fast enough for even time sensitive enzymatic assays. Furthermore, the system demonstrated excellent linearity in a demanding biological assay application.