

Biomek® NX

MICROLITER AND SUB-MICROLITER WET-WELL PIPETTING WITH THE BIOMEK MULTICHANNEL AP96-CHANNEL DISPOSABLE TIP HEAD

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Introduction

The Biomek NX Multichannel Laboratory Automated Workstation, the newest liquid handler offered by Beckman Coulter, Inc., was introduced in 2004 with capabilities for 96- and 384-channel pipetting. When equipped with a 96-channel head the system can pipette into 96- and 384-well plates as well as many other specialized plates including protein crystallization and MALDI plates. What makes the Biomek NX Multichannel unique is its versatility and expandability to meet the ever-changing needs in the Life Science Research market. The Biomek NX Multichannel is capable of pipetting in a variety of specific applications such as nucleic acid preparation, plate replication and assay plate preparation.

This technical information bulletin provides details that characterize microliter and sub-microliter wet-well pipetting performance of the Biomek NX Multichannel fitted with a 96-Channel Disposable Tip Head. The experiments were conducted using a Fluorescein and Trizma* buffer solution to mimic typical plate replication and HTS applications. This multichannel head was designed to use either AP96 P20 or P250 disposable tips that employ the industry standard of 9-mm center spacing. For this work AP96 P250 tips were used.



Figure 1. The Biomek NX Multichannel liquid handler equipped with a 96-Channel Disposable Tip Pipetting Head and a Stacker Carousel.

Manufacturing Criteria

Beckman Coulter has established a set of specifications that each head must meet in a manufacturing test before shipment. For the Biomek 96-Channel Disposable Tip Pipetting Head (200 μ L) the criteria is a transfer of 200 μ L into a wet-well at <1.00% C.V. and a transfer of 5 μ L at <2.00% C.V using P20 tips. The tests are performed as follows: 5 μ L or 200 μ L of Eosin Y dye solution is dispensed into three 96-well microplates and read on the microplate reader for an immediate read at 510 nm and 650 nm, to account for background. The percent CV of each plate must fall below the current specification to qualify.



Testing Beyond the Specifications

It is understood that the Biomek NX will be used in the laboratory to pipette a wide variety of liquids at lower volumes, far too many to include in the manufacturing test. Thus, the experiments described in this bulletin were carried out to characterize the performance of the systems under typical assay conditions used in the research laboratory.

The study conducted tested the pipetting capabilities at 5.0-, 1.0- and 0.5 μL . This evaluation was performed on a Biomek NX Multichannel equipped with the 96-Channel Disposable Tip Pipetting Head. Biomek AP96 P250 tips were used in this study.

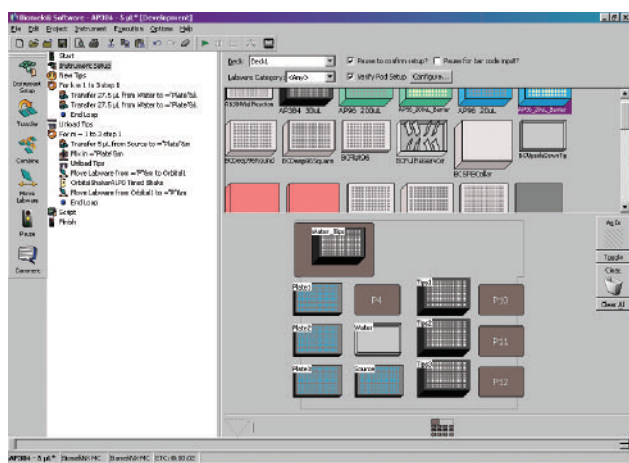


Figure 2. The Biomek NX method used for microliter and sub-microliter pipetting created with Biomek Software (v 3.1).

Solutions and Techniques Used

A Biomek NX method (Figure 2) was created that dispenses a fluorescein and Trizma buffer (Catalogue No. 203-2A) solution into 96-well flat-bottom microplates (Greiner Catalog No. 655201). Fluorescein dye stock solutions were made by adding varying quantities of Fluorescein to Trizma buffer to obtain fluorescence readings at wavelengths from 485 to 538 for each volume pipetted. For each volume, the raw data results of three plates were taken in total and the CV across all wells was reported.

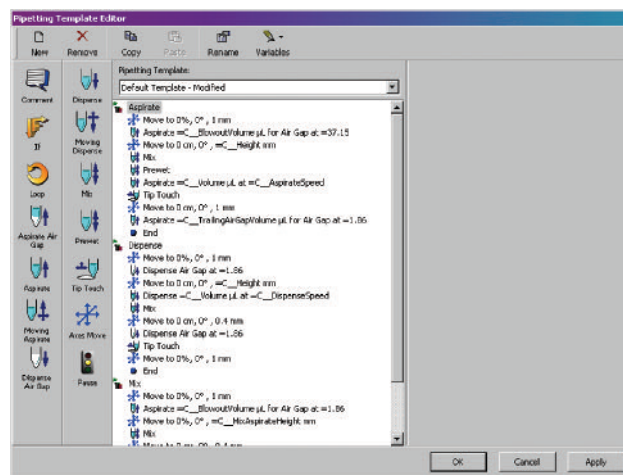


Figure 3. Pipetting template created for microliter and sub-microliter pipetting.

A new pipetting template (Figure 3) was created which employed a dispense of the sample volume and 1.86 μL of the blowout volume at the center of the well. Individual calibration values for Slope and Offset were set to adjust the accuracy to meet a standard of less than $\pm 3\%$ difference from the desired dispense volumes. The calibration values were determined and accuracy requirements were achieved by the methods described in a separate Technical Information Bulletin, "Improving Accuracy by Use of Technique Calibration"¹.

The total volume of each well in the microplates was normalized to 100 μL with Trizma buffer. For example, the 1.0 μL assay was performed by dispensing 99 μL of Trizma buffer to each well of three empty 96-well flat-bottom microplates. Then 1.0 μL of Fluorescein was added with the 96-Channel Disposable Tip Head to each plate and the contents of the well were mixed. The plates were then transported using the grippers on the instrument to the Orbital Shaker ALP (Automated Labware Positioner) for a two-minute shake. Lastly, the plate was taken to the Molecular Devices SPECTRAMax* GeminiXS plate reader for an immediate read at 485 nm and 538 nm (to account for background).

The Biomek NX method incorporated the following parameters in the Technique for Fluorescein dispense:

Speed within the well	5% (Set in the custom Pipetting Template to 5%)	Follow liquid level	
Aspiration Height	-1 mm from Liquid	Aspirate	Yes
Dispense Height	-1 mm from Liquid	Dispense	Yes
Aspiration		Tip Touch	
Trailing Airgap	0 µL	Aspirate	No
Delay	1250 ms	Dispense	No
Speed	29.72 µL/s	Prewet Tips	Yes
Blowout		Mix prior to Aspirate	No
Volume	10 µL	Trailing Air Gap	Yes
Delay	0 ms	Blowout Leading Air Gaps	Yes
Dispense		Mix after Dispense	No
Delay	0 ms		
Speed	29.72 µL/s		

The Results of this Study

Three plates were run on a 96-Channel Disposable Tip Head at each volume. The values reported in Table 1 represent the % C.V. across all wells.

Volume	%CV
5.0 µL	1.95%
1.0 µL	3.77%
0.5 µL	12.04%

Table 1. Results of microliter and sub-microliter wet-well pipetting experiments.

Conclusion

This bulletin shows the typical pipetting performance of the Biomek NX Multichannel with 96-Channel Disposable Tip Head when performing a common laboratory application. End-user results will likely vary when using reagents and labware other than those described. However, the Biomek Software is rich in pipetting control features that should enable most laboratories to obtain similar results for their respective application.

References

1. *Improving Accuracy by Use of Technique Calibration. Technical Information Bulletin T-1915A, Beckman Coulter, Inc.*

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