

Automating the C100_{HT} Biologics Analyzer Sample Preparation on a Biomek i5 Liquid Handler Platform

Mervin Gutierrez, Tingting Li, and Marcia Santos
SCIEX Separations, Brea, CA

Introduction

There is an increasing need in the biopharmaceutical industry for large-scale N-glycosylation profiling of therapeutic antibodies in all phases of product development. This need is highlighted particularly during clone selection and cell culture optimization where hundreds of samples should be analyzed in a short period of time to properly assess their glycosylation profiles.

The SCIEX C100_{HT} Biologics Analyzer is a high throughput glycan screening platform consisting of a pre-filled multi-capillary cartridge enabling end users to process hundreds of samples per day.¹ C100_{HT} glycan sample preparation (digestion, labeling and clean up) is a magnetic bead—mediated process eliminating lengthy, manual centrifugation and vacuum—centrifugation steps found in traditional protocols.

This technical note describes the steps necessary for automation of C100_{HT} sample preparation workflow using MAK33, a purified recombinant monoclonal antibody.

Key Features

- Reproducible high throughput screening of glycans
- Automated sample preparation
- Step by step automation instructions

Materials and Methods

Chemical and Reagents

Water and acetonitrile was obtained from Burdick-Jackson. The purified recombinant monoclonal antibody MAK33 was obtained from Roche Diagnostics (Indianapolis, IN) diluted to 10mg/mL before sample preparation. PNGase F was obtained from New England Biolabs (Boston, MA). 2-picoline-borane (pic-BH₃) and DMSO were obtained from Sigma-Aldrich (St Louis, MO).



SCIEX C100_{HT} Biologics Analyzer

Reagents used to prepare the denaturation, digestion, and labeling master mix solutions were provided in the chemistry kit (SCIEX, PN C13787). The SCIEX C100_{HT} Biologics Analyzer is equipped with LED-induced fluorescence (LED-IF) detection capability with excitation at 465 nm and emission at 540 nm, allowing for the detection of 1-aminopyrene-3,6,8-trisulfonate (APTS) labeled N-glycans.

Master Mix Preparation

Denaturation solution mix, Digestion solution mix, and Labelling solution mix were prepared using the reagent volumes in Table 1, Table 2 and Table 3. The Labeling solution mix was protected from exposure to light until the labeling step.

Reagent	For 1 Sample +15% dead volume	For 96 Samples +15% dead volume
Reconstituted D1	1.15 μ L	111 μ L
Reconstituted D2	1.15 μ L	111 μ L
D3	1.15 μ L	111 μ L
D4	5.75 μ L	552 μ L

Table 1. Denaturation solution.

Reagent	For 1 Sample +15% dead volume	For 96 Samples +15% dead volume
D4	13.8 μ L	1325 μ L
PNGase F enzyme	1.15 μ L	111 μ L

Table 2. Digestion solution.

Reagent	For 1 Sample +15% dead volume	For 96 Samples +15% dead volume
Reconstituted APTS-30	11.5 μ L	1104 μ L
D4	5.75 μ L	552 μ L
Reductant Solution	1.15 μ L	111 μ L
Reconstituted IST	1.15 μ L	111 μ L
Analytical Grade Water	9.2 μ L	884 μ L

Table 3. Labeling solution.

Automation for Large-Scale Sample Processing

Automated sample preparation was performed using a Biomek i5 Series Laboratory Automation Workstation from Beckman Coulter (Indianapolis, IN), which was set up with 96-well plate holders, a 96-well plate magnet and stand, and 230 μ L and 50 μ L pipette tips and holders. The Biomek was also equipped with a 300 μ L 96 pipetting head, a heat block, an orbital shaker and a tip washing station. The Biomek Control Software was version 4.0 (Beckman Coulter). Figure 1 illustrates the deck set up of the Biomek and Table 4 lists the labware used for this sample preparation.

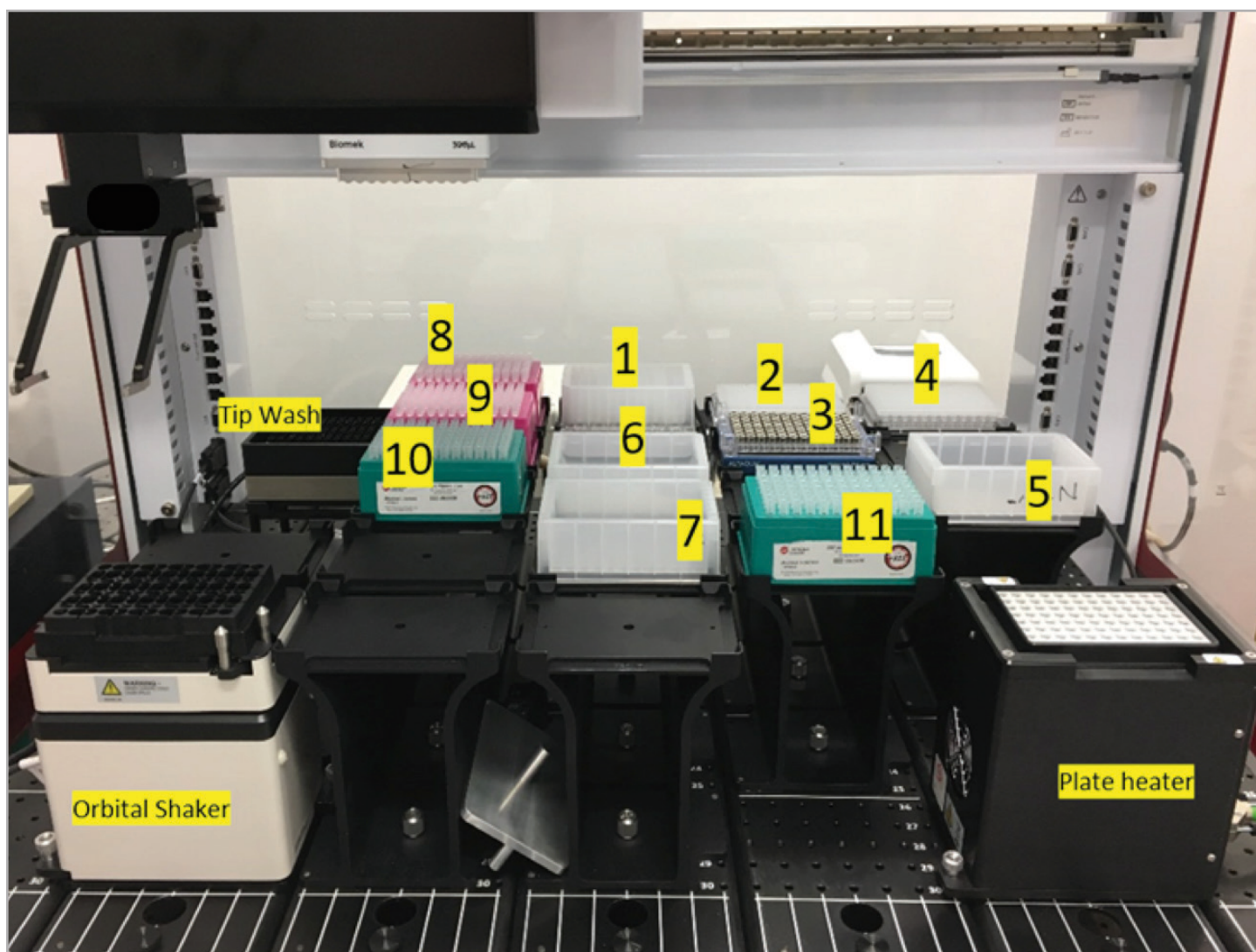


Figure 1. Deck setup on the Biomek i5 Laboratory Automation Workstation.

Number	Labware
1	Reagent source plate
2	Biomek sample preparation plate
3	Alpaqua magnetic plate (A000380)
4	Biomek sample elution plate for analysis on C100HT
5	Acetonitrile reservoir (Agilent, PN 201244-100)
6	DDI water reservoir

Number	Labware
7	D4 reservoir
8	50 µL tip box (Beckman Coulter, PN B85888)
9	50 µL tip box
10	230 µL tip box (Beckman Coulter, PN B85906)
11	230 µL tip box

Table 4. Labware used for glycan analysis automation.

Results and Discussion

The glycan sample preparation workflow is shown in Figure 2. Briefly, the sample is denatured to allow for the endoglycosidase effective cleaving reaction. The following steps consist of endoglycosidase digestion using PNGase F performed at 60° C for 20 min. Upon completion of the digestion step, the N-released glycans are captured using magnetic beads by adding acetonitrile to a final concentration of 90% in the reaction medium. The APTS labeling reaction is performed via reductive amination of the glycans while still associated with the magnetic

beads. Like in the deglycosylation reaction, the glycan labeling step is also performed at 60° C for 20 min. Excess APTS removal is performed by adding acetonitrile to the reaction mixture to yield 90% final concentration which is necessary for capture the glycans by the beads. For high-efficiency APTS dye removal, the beads were washed three times in every step, first with water for easy and fast resuspension, followed by acetonitrile to yield 90% final concentration for glycan recapture. The APTS labeled glycans were then eluted from the beads by the addition of 50 μ L water and were ready for analysis on C100HT.



Figure 2. C100HT glycan sample preparation workflow.

Automation Steps (Simplified)

- Initially 200 μ L of magnetic bead suspension is added using manual multichannel pipettes to the sample plate and the plate is placed on the deck (Fig. 1, position 2).
- The sample plate is moved to magnetic plate (Fig. 1, position 3) for incubation for 90 seconds and the bead storage solution is aspirated.
- The sample plate is removed from deck. 10 μ L of the sample solution (preferred concentration: 10 mg/ml) is dispensed in each well of the 96 well plate followed by the addition of 5 μ L of the Denaturing solution (Fig. 1, position 2).
- The Biomek moves the sample plate to the orbital shaker and the samples is mixed for 30 sec. The plate is then moved to the heating block and the sample is incubated for 8 min at 60° C.
- After the denaturation step is completed, 12 μ L of the digestion solution is added to each well (Fig. 1, position 2).
- The deglycosylation reaction is implemented by heating the plate at 60° C for 20 min.
- Upon completion of the digestion step, acetonitrile (Fig. 1, position 5) is added to each sample for a 90% final acetonitrile concentration. During this step, the glycans are captured by the beads.
- After glycan capture, the plate is transferred to the magnetic stand (Fig. 1, position 3) and the supernatant is removed.
- The plate is moved back to (Fig. 1, position 2) and 25 μ L of the labeling solution is dispensed in each well.
- The plate is shaken for 30 sec. on the orbital shaker to allow for proper mixing of the beads and the labeling reaction. The plate is moved to the heating plate and the last incubating step takes place at 60° C for 20 min.
- Upon completion of the labeling reaction the plate returns to position 2 and 10 μ L of reagent D4 (Fig. 1 position 6) is dispensed in each well and the plate is moved to the orbital shaker for the mixing.
- 160 μ L of acetonitrile (Fig. 1 position 5) is dispensed in each well and mixed with the beads by pipetting the volume up and down 5 times at the slowest speed available.
- Step 10 and 11 are repeated 2 more times except that the 10 μ L of reagent D4 is replaced by 20 μ L of ddi water.
- The APTS-labeled glycans are eluted from the beads by dispensing 50 μ L of ddi Water (Fig. 1, box 7) in each well and by pipetting the volume up and down 5 times at the slowest speed available. The plate is placed onto the magnetic stand the eluent is transferred to a clean plate (Fig. 1, box 4).

Robustness of the Automated Sample Preparation Process and CE-LIF Analysis

Figure 3 shows a representative electropherogram for the MAK33 antibody drug product. Two plates were prepared using the workflow described in the previous section.

To assess the reproducibility of automated sample preparation, two 96-well plates were prepared. Glycans released from MAK33 were analyzed for corrected peak area % between each sample well for G0, G0F, G1F, G1'F and G2F species. The data shown in Figure 4 illustrates variability across two 96-well plates (192 individually labeled MAK33 samples) using the BioMek i5.

Some variability is expected for individually prepared samples, especially for relative peak areas close to or less than 5% due to challenge in integrating peaks close to the baseline. However, %RSD of the most major glycan species (G0F, G1F and G1'F) for percent corrected peak area remains low at less than 5% RSD. Implementing automation of deglycosylation, APTS labeling and cleanup using of the Biomek i5 Liquid Handler reduces the need for human intervention while providing better precision of pipetting to minimize variation. Subsequent downstream glycan data analysis on the C100_{HT} provides the tools needed for easy glycan identification and reporting.¹

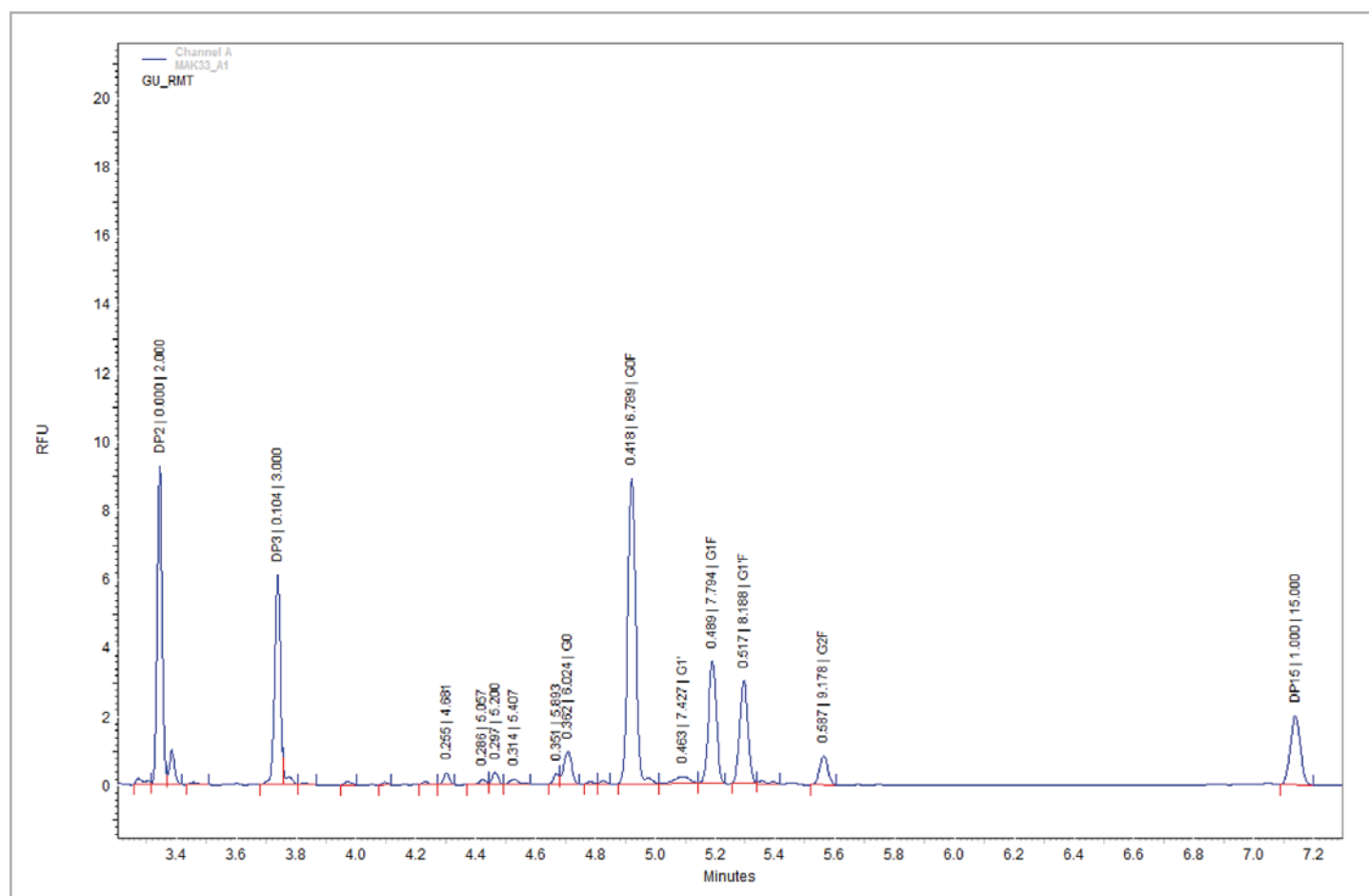


Figure 3. Representative electropherogram of MAK33 sample on C100_{HT}.

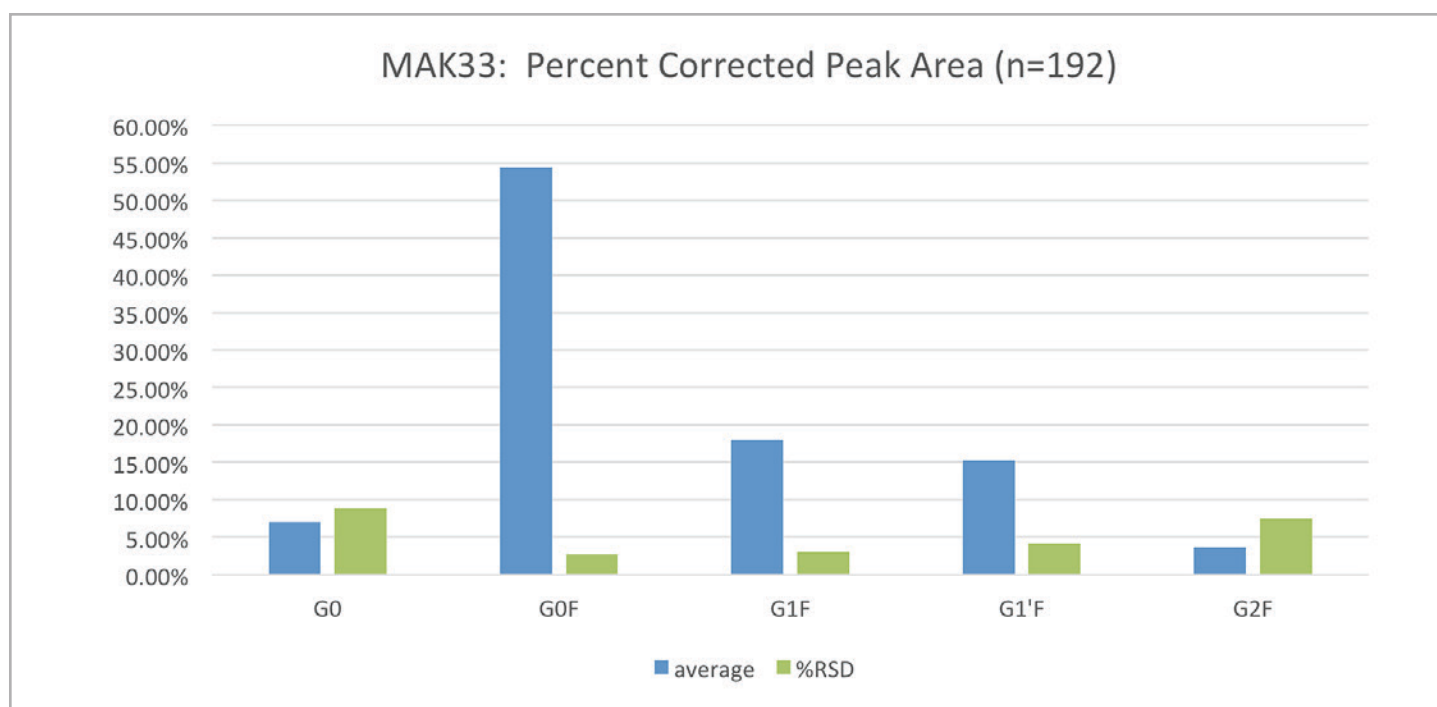


Figure 4. Average of corrected area percent of 5 major glycans found in MAK33 sample on C100_{HT} and respective %RSD for 192 samples.

Conclusion

In this technical note, a new automated sample preparation protocol for analysis on C100_{HT} is presented. Automation of the C100_{HT} glycan sample preparation using the Biomek i5 greatly simplified the process by minimizing human intervention

and variability that may occur as a result reducing the sample preparation time from approximately 3 hours for a full 96 well plate to 1 hr and 45 min using the Biomek i5.

Supplemental Material – Step by Step Automated Sample Preparation

- Step 1. Prepare the deck of the Biomek i5 according to Figure 1.
- Step 2. Transfer 200 μ L of the magnetic bead suspension into each sample well of SPP (sample preparation plate).
- Step 3. Move SSP to magnetic stand and wait for 90 sec.
- Step 4. Aspirate supernatant to remove the storage solution from the beads.
- Step 5. Move SSP to position 2.
- Step 6. Dispense 100 μ g of the antibody sample in each well.
- Step 7. Move SSP to orbital shaker and shake plate for 30 sec. at 1500 rpm.
- Step 8. Move SSP to position 2.
- Step 9. Dispense 5 μ L of Denaturation Solution to each well.
- Step 10. Move SSP to orbital shaker and shake plate for 30 sec. at 1500 rpm.
- Step 11. Move the Biomek Sample Preparation Plate to the on-deck heating plate and incubate Biomek Sample Preparation Plate at 60° C for 8 min.

- Step 12. The method will pause and request to dispense the digestion solution into each well of Biomek Sample Preparation Plate.
- Step 13. Remove the plate from deck and use a single channel pipette to add 12 μL of Digestion solution into each well.
- Step 14. Put the well back on position P6 of the deck and click OK on the previous pop-up window for the method to continue to the next step: deglycosylation.
- Step 15. The Biomek will move the Biomek Sample Preparation Plate to the on-deck orbital shaker and shake the plate at 1200 RPM for 30 sec.
- Step 16. Next the Biomek Sample Preparation Plate will move to the heating plate where it will incubate at 60° C for 20 min.
- Step 17. After the deglycosylation step, move Biomek Sample Preparation Plate to an orbital shaker and shake for 30 sec. at 1200 RPM.
- Step 18. Move the Biomek Sample Preparation Plate to the P6 position on the deck.
- Step 19. Transfer 200 μL of acetonitrile into each sample well (this step will result in approximately 90% final acetonitrile concentration in the vial) for glycan capture. Using the slow speed and the pipetting mix technique to slowly pipette the sample volume up and down 5 times.
- Step 20. Place the Biomek Sample Preparation Plate onto the magnetic separator and wait for 90 seconds. Remove the supernatant (approximately 240 μL) from the sample well by careful and slow pipetting from the bottom of the vial twice by removing small volumes.
- Step 21. Remove the Biomek Sample Preparation Plate from the magnetic separator.
- Step 22. Put the Biomek Sample Preparation Plate on the home position.
- Step 23. The method will pause to add the labeling solution.
- Step 24. Remove the Biomek Sample Preparation Plate from the deck and put it on the bench top.
- Step 25. Using a single channel pipette, transfer 25 μL of the previously prepared Labeling Solution into each well.
- Step 26. Place the Biomek Sample Preparation Plate on the deck and click OK on the previously pop-up window to resume the method.
- Step 27. The Biomek will move the Biomek Sample Preparation Plate to the on-deck orbital shaker and shake SPP for 30 sec. at 1200 RPM.
- Step 28. Move the Biomek Sample Preparation Plate to the on-deck heat block and incubate the plate at 60° C for 20 min. (Labeling Step).
- Step 29. After the completion of the labeling step, the Biomek will proceed with the sample clean up comprised of the following steps:
- Step 30. Move the Biomek Sample Preparation Plate to the deck's home position.
- Step 31. Dispense 10 μL of DDI water into each well of the Biomek Sample Preparation Plate.
- Step 32. Move the Biomek Sample Preparation Plate to the on-deck shaker and shake the plate for 30 sec. at 1200 rpm.
- Step 33. Move the Biomek Sample Preparation Plate to the home position.
- Step 34. Dispense 160 μL of Acetonitrile on to the Biomek Sample Preparation Plate and pipette up and down at a low speed to mix the sample volume 5 times.

- Step 35. Move the Biomek Sample Preparation Plate to the magnetic plate.
- Step 36. Wait 180 sec. or until all beads are gathered on the walls of the wells.
- Step 37. Slowly pipette out approximately 180 μ L of solution from Biomek Sample Preparation Plate.
- Step 38. Move Biomek Sample Preparation Plate to the home position.
- Step 39. Dispense 20 μ L of DDI water on to the Biomek Sample Preparation Plate.
- Step 40. Move the Biomek Sample Preparation Plate to the on-deck shaker and shake the plate for 30 sec. at 1200 rpm.
- Step 41. Move the Biomek Sample Preparation Plate to the home position.
- Step 42. Dispense 160 μ L of Acetonitrile on to the Biomek Sample Preparation Plate.
- Step 43. Pipette up and down at a low speed to mix the sample volume 5 times.
- Step 44. Move the Biomek Sample Preparation Plate to the magnetic plate.
- Step 45. Wait 240 sec. or until all beads are gathered on the walls of the wells.
- Step 46. Slowly pipette out approximately 180 μ L of solution from Biomek Sample Preparation Plate.
- Step 47. Move Biomek Sample Preparation Plate to the home position.
- Step 48. Dispense 20 μ L of DDI water on to the Biomek Sample Preparation Plate.
- Step 49. Move the Biomek Sample Preparation Plate to the on-deck shaker and shake the plate for 30 sec. at 1200 rpm.
- Step 50. Move the Biomek Sample Preparation Plate to the home position.
- Step 51. Dispense 160 μ L of Acetonitrile on to the Biomek Sample Preparation Plate.
- Step 52. Pipette up and down at a low speed to mix the sample volume 5 times.
- Step 53. Move the Biomek Sample Preparation Plate to the magnetic plate.
- Step 54. Wait for 240 sec. or until all beads are gathered on the walls of the wells.
- Step 55. Pick up 180 μ L of solution from Biomek Sample Preparation Plate.
- Step 56. Move Biomek Sample Preparation Plate to the home position.
- Step 57. Dispense 50 μ L of DDI water onto the Biomek Sample Preparation Plate.
- Step 58. Using same pipette tip, pipette up and down at a low speed to mix the sample volume 5 times.
- Step 59. Move the Biomek Sample Preparation Plate to the on-deck shaker and shake the plate for 30 sec. at 1200 rpm.
- Step 60. Move the Biomek Sample Preparation Plate to the magnetic plate.
- Step 61. Wait 240 sec. or until all beads are gathered on the walls of the wells.
- Step 62. Slowly pipette out approximately 50 μ L of solution from Biomek Sample Preparation Plate.
- Step 63. Transfer the 50 μ L eluate to the C100_{HT} sample plate.
- Step 64. End of automated sample preparation method.

References

1. M. Lies, T. Li, M. Santos, High-Throughput Glycan Screening to Remove Biopharmaceutical Development Bottlenecks, Technical Note RUO-MKT-02-7361-A
2. L.R. Ruhaak, G. Zauner, C. Hunn, C. Bruggink, A.M. Deelder, M. Wuhrer; *Anal. Bioanal. Chem* 397(2010)3457-3481

Who is SCIEX? SCIEX company's global leadership and world- class service and support in the capillary electrophoresis and liquid chromatography-mass spectrometry industry have made it a trusted partner to thousands of the scientists and lab analysts worldwide who are focused on basic research, drug discovery and development, food and environmental testing, forensics and clinical research.

Contact Us: [sciex.com/contact-us](https://www.sciex.com/contact-us)

For Research Use Only. Not for use in diagnostic procedures.

© 2018 AB SCIEX. SCIEX is part of AB SCIEX. The trademarks mentioned herein are the property of AB Sciex Pte. Ltd. or their respective owners. AB SCIEX™ is being used under license. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA.

Publication number: RUO-MKT-02-8018-A 07/2018



Headquarters

500 Old Connecticut Path, Framingham, MA 01701, USA
Phone 508-383-7800
[sciex.com](https://www.sciex.com)

International Sales

For our office locations please call the division headquarters or refer to our website at
[sciex.com/offices](https://www.sciex.com/offices)