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Mid-Scale Serial Dilution with Mammoth™ 30mL Automation Tips on the Lynx® Platform

Abstract

Mid-scale liquid handling workflows often require the controlled transfer of liquids from a source vessel into one or more destination vessels while maintaining controlled volume transfer and mixing. The Mammoth™ 30mL Automation Tip for the Dynamic Devices Lynx® platform supports transfer and distribution of mid-scale liquid volumes and distribution of mid-scale liquid volumes (1.5-20mL) between reservoirs, tubes, and multiwell plates, closely mimicking manual serological pipetting while reducing plastic consumption and simplifying automated methods.

This application note describes serial dilution workflows using Mammoth 30mL Automation Tips on the Lynx platform, including recommended operating parameters and volume considerations for generating mid-scale serial dilution series in 6-well and 24-well plate formats.

Introduction

Serial dilution workflows are commonly used for media optimization, reagent titrations, cell culture studies, and assay development. When working with larger volumes in 6-well and 24-well plates, traditional 1mL and 5mL automation tips often require multiple aspiration and dispense cycles to complete each dilution step. This increases method complexity, extends run times, and can introduce variability into the dilution process.

The Mammoth™ 30mL Automation Tip allows large-volume diluent additions, mixing, and well-to-well transfers to be performed with a single tip, functioning similarly to an automated serological pipette. This approach simplifies method development and can reduce tip consumption compared with workflows that rely on multiple smaller-volume tips.

This application note focuses on generating serial dilution series directly in 6-well and 24-well plates for cell culture, reagent preparation, and process development applications.

Product Features

- Supports single-stroke aspiration and dispensing of 1.5-20mL volumes for mid-scale serial dilution workflows.
- Enables diluent addition, mixing, and well-to-well transfers using a single Mammoth™ 30mL Automation Tip.
- Simplifies serial dilution method development in 6-well and 24-well plate formats.
- Supports consistent dilution workflows through high-volume liquid handling.
- Reduces aspiration and dispense cycles compared to workflows using smaller-volume automation tips.
- Sterile, filtered tip design suitable for cell culture and reagent preparation applications.
- Includes 5mL graduation marks for visual volume verification.
- Compatible with Dynamic Devices Lynx LM series liquid handlers equipped with 24-channel ST or VVP pipetting arms.

Required Materials

- Dynamic Devices Lynx LM series liquid handler with 24-channel pipetting arm.
- Mammoth 30mL Automation Tips (PN 9453030).
- Thomson Tip Rack Adapter (PN 1212633-399).
- Thomson 6-well plates and 24-well plates (e.g., PN 931167, 931170, 931565-G-1X, 931568, 931569-G-1X, 931571) or similar.
- Thomson Universal 4-Position Rack (PN 1212605-399).
- Standard reagent reservoirs (high profile, approximately 300mL).
- Water, PBS, or 20% glycerol solution for setup and verification.

Representative Source and Destination Vessel Combinations

Source Vessel	Destination Vessel	Typical Application
50mL Conical Tube	6-Well Plate	Cell culture seed trains, media optimization studies, process development
50mL Conical Tube	24-Well Plate	Reagent titrations, screening assays, dilution series generation
Reagent Reservoir	6-Well Plate	Buffer addition, media preparation, large-volume dilution workflows
Reagent Reservoir	24-Well Plate	Assay preparation, media exchange, reagent distribution
6-Well Plate	6-Well Plate	Transfer and expansion of dilution series between culture plates
6-Well Plate	24-Well Plate	Scaling studies, culture transfer, assay preparation

Table 1: Representative Source and Destination Vessel Combinations for Mid-Scale Serial Dilution Workflows. Suitability depends on vessel geometry, working volume, and liquid handling requirements.

For a true 2-fold dilution:

- Add 4mL diluent to each receiving well
- Transfer 4mL from the previous well
- Mix to a total volume of 8mL
- Transfer 4mL to the next well
- Retain 4mL in the current well

Well	Media or Diluent	Stock to Transfer In	Suggested Mixing Volume (50%)	Transfer Out	Final Volume Remaining	Relative Concentration
A1	4mL	4mL Stock	4	4mL	4mL	1:2
A2	4mL	4mL from A1	4	4mL	4mL	1:4
A3	4mL	4mL from A2	4	4mL	4mL	1:8
A4	4mL	4mL from A3	4	4mL	4mL	1:16
A5	4mL	4mL from A4	4	4mL	4mL	1:32
A6	4mL	4mL from A5	4	0mL*	8mL	1:64

Table 2a: True 2-Fold Serial Dilution in a 24-Well Plate (4mL Final Volume Retained per Well) *Discard 4mL from A6 if all wells must contain exactly 4mL.

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Well	Media or Diluent	Stock to Transfer In (mL)	Suggested Mixing Volume (50%)	Transfer Out	Final Volume After Transfer	Relative Concentration
A1	20mL	20 Stock	20	20mL	20mL	1:2
A2	20mL	20 from A1	20	20mL	20mL	1:4
A3	20mL	20 from A2	20	0*mL	40mL	1:8

Table 2b: True 2-Fold Serial Dilution in a 6-Well Plate *No transfer from the final well; discard 20mL from A3 if all wells must contain exactly 20mL.

Parameter	24-Well Plate Workflow	6-Well Plate Workflow
Retained Volume per Well	4mL	20mL
Transfer Volume	4mL	20mL
Total Mixing Volume	8mL	40mL
Number of Dilution Steps	6	3
Typical Application	Assay development, media screening, reagent titration	Process development, media optimization, cell culture studies
Mammoth Transfer Compatibility	Workflow status	Demonstrated

Table 2c: Comparison of 2-Fold Serial Dilution Workflows Using Mammoth™ 30mL Automation Tips.

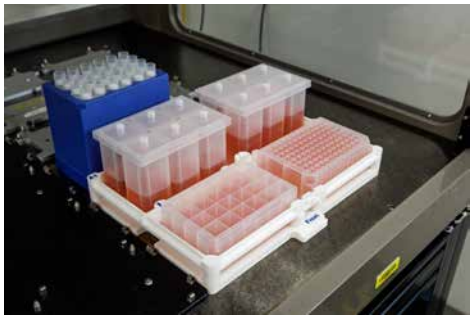

Figure 1: Mid-scale serial dilution deck configuration using 6-well plates, 24-well plates, and reagent reservoirs.

Overview

- **Source:** concentrated reagent or cell suspension in 6-well plates or other appropriate source container.
- **Diluent:** Buffer or media in a 6-well plate or other reservoir.
- **Destination:** 24-well or 6-well plate for mid-scale assay or culture.

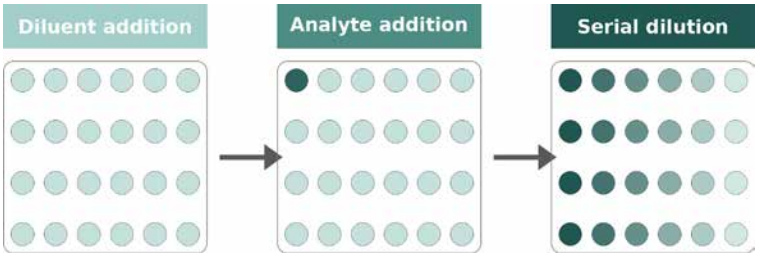

Figure 2: Serial dilution workflow overview — diluent addition, analyte addition, and serial dilution across the plate.

Schematic

1. Aspirate diluent from a reagent reservoir using the Mammoth™ 30mL Automation Tip.
2. Dispense the diluent into a series of wells in a 6-well or 24-well plate.
3. Add the starting concentration to the first well of the dilution series.
4. Mix the first well by aspirating and dispensing at least 50% of the working volume.
5. Transfer the defined dilution volume to the next well and repeat the mix-and-transfer process across the plate to generate the desired dilution series.

Method

1. **Pre-fill diluent wells.** Use offset tip loading to load 2 tips for a horizontal serial dilution or 3 tips for a vertical serial dilution. Aspirate the desired total diluent volume (for example, 15,000µL total, 3,000µL per well for five wells), then multi-dispense into the 24-well plate (e.g., A1 → A2 → A3 → A4 → A5) using a west-to-east dispense pattern.

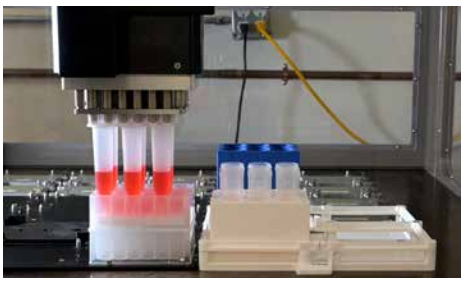

Figure 3: Aspiration from 50mL conical tubes for transfer into a 24-well plate.

2. **Add stock to the first dilution well.** Aspirate the calculated stock volume from a source vessel (for example, 3mL). Dispense into the first well of the series (e.g., A1) and perform three mixing cycles with an appropriate bottom offset, aspirating at least half the well volume.
3. **Serially transfer between wells.** From the mixed A1 well, aspirate the transfer volume (e.g., 3mL) and dispense into A2; mix as in the previous step. Repeat for A2 → A3 and A3 → A4, or adjust the pattern to match the desired dilution series.

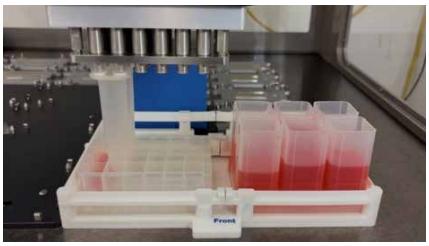

Figure 4: serial transfer between wells.
Figure 5: resulting concentration gradient across the dilution series.

Well	Diluent (mL)	Stock / prev. well (mL)	Final (mL)	Notes
A1	6.0	3.0 Stock solution	6.0	mix 4.5mL total (50% of 9.0mL) transfer 3.0mL to A2
A2	6.0	3.0 from A1	6.0	mix 4.5mL total (50% of 9.0mL) transfer 3.0mL to A3
A3	6.0	3.0 from A2	6.0	mix 4.5mL total (50% of 9.0mL) transfer 3.0mL to A4

Well	Diluent (mL)	Stock / prev. well (mL)	Final (mL)	Notes
A4	6.0	3.0 from A3	6.0	mix 4.5mL total (50% of 9.0mL) transfer 3.0mL to A5
A5	3.0	3.0 from A4	6.0	Final well; no transfer out, 6.0mL retained

Table 4. 3-Fold, Five-Point Serial Dilution Workflow in a 24-Well Plate.

Notes and Best Practices

- Use "Mid Speed Slow Enter/Exit" for aspiration and dispense to maintain precision at higher volumes.
- Include a post-airgap (25–50µL) to prevent droplet formation during plate moves.
- Validate accuracy initially with colored water or PBS before introducing critical reagents.

Choosing the Right Volume Range

The Thomson Mammoth 30mL Automation Tip is designed for reliable aspiration and dispensing in the 1.5–20mL range (nominal 30mL tip volume).

Volume Selection Guidelines

- **Minimum transfer volume:** 1.5mL (5% of nominal volume).
- **Mixing volume:** at least 50% of total well volume for thorough homogeneity.

Well	Media or Diluent	Stock to Transfer In (mL)	Suggested Mixing Volume (50%)	Transfer Out	Final Volume After Transfer	Relative Concentration
A1	19.8mL	2.2 Stock	11.0	2.2mL	19.8mL	1:100
A2	19.8mL	2.2 from A1	11.0	2.2mL	19.8mL	1:1000
A3	19.8mL	2.2 from A2	11.0	0mL	22.0mL	1:10000

Table 3. Example 10-Fold Serial Dilution Workflow in a 6-Well Plate. *No transfer from the final well.

Each well contains 19.8mL of diluent and receives a 2.2mL transfer from the preceding well. The resulting 22mL mixture is mixed thoroughly, and 2.2mL is transferred to the next well to create a true 10-fold dilution series. This workflow generates concentrations of 10^{-1}, 10^{-2}, and 10^{-3} while maintaining a 22mL working volume in each well and using transfer volumes compatible with Mammoth™ 30mL Automation Tips.

Workflow: Mid-Scale Serial Dilution

This workflow uses a single Mammoth 30mL tip to generate a mid-scale serial dilution series in a 24-well or 6-well plate.

Critical Height and Timing Parameters for Lynx Automation

Understanding liquid-level tracking and aspiration timing is essential for preventing air aspiration and ensuring consistent volume transfers.

Height Definitions

- **"Starting Height"** – height in millimeters up from the Z-zero position of the labware deck location.
- **"Final Height"** – also height in millimeters up from the Z-zero position of the labware deck location.

Volume-to-Height Relationships

- **6-well plate:** every 16,000µL (16mL) aspirated ≈ 9mm height difference.

Recommended Aspiration Speed and Timing

- 6-well plate @ 1000µL/s: for every 9mm of downward travel, allow 8s (9mm : 8s).
- 6-well plate @ 2000µL/s: 9mm : 5s.
- 6-well plate @ 3000µL/s: 9mm : 4s.

Example: aspirating 16mL from a 6-well plate at 3,000 µL/s drops the liquid level 9 mm and should take approximately 4 s. Set tip travel to track from the starting height to (starting height 9 mm).

Practical Application

When programming workflows, use these ratios to:

1. Calculate "Starting Height" based on initial fill volume.
2. Calculate "Final Height" based on the volume to be aspirated.
3. Set aspiration speed to match the distance:time ratio.
4. Prevent tips from aspirating air due to premature completion.

These parameters help prevent the following errors:

- Tips moving faster than liquid can enter (causes air gaps).
- Tips reaching final position before aspiration completes (causes air aspiration).
- Inconsistent volumes due to varying liquid-column heights.

Troubleshooting and Tips

Issue	Likely cause	Recommended action
Tips cannot clear tip box after loading	Z height too low	Reteach labware Z; use 30mL Tip Tray Adapter
Tips leak when leaving liquid	Tips not loaded onto the pipetting arm properly	After tip loading, check motor current; it should read above 15%
Tips fall off after loading	Tip loading depth incorrect	Reteach the tip-loading height and confirm complete tip seating before rerunning.

Table 5. Troubleshooting guidelines for mid-scale serial dilution workflows using Mammoth™ 30mL Automation Tips.

Conclusion

The Mammoth™ 30mL Automation Tip supports mid-scale serial-dilution workflows in 6-well and 24-well plates on the Dynamic Devices Lynx platform. By enabling large-volume transfers and mixing, it can reduce aspiration and dispense cycles while simplifying method development. The workflows described in this application note provide a practical approach for generating serial dilution series.