**Best Practices: Dispensing Cells and Maintenance of Multidrop Dispensing Cassettes**

**Cell Dispensing – Helpful Tips in Maximizing Results**
To optimize and get the best results from your Multidrop Combi dispenser, here are some guidelines to get the best performance out of the instrument and the cassettes.

**Choose the Correct Cassette**
Ensure that the cells can properly be dispensed without damage, i.e. shearing, while passing through the opening of the tip. It is recommended to use the standard tubing cassette for plating cells. Small tubing cassettes can be used for small sized cells, but the larger tips are less prone to clogging. If using the small tubing cassettes, it is recommended to start with the plastic tips since the metal version sometimes affects the charge on the cell membrane.

**Autoclave Sterilization**
The most common cause of inconsistent cell dispensing is clogging, and the most common cause of clogging is using alcohol to rinse the tubings. Alcohols such as ethanol and isopropanol precipitates proteins. If ethanol is used for washing the tubing, there is a need to rinse the tubing with large amounts of water. However, this puts unnecessary wear on the tubings. It is recommended to autoclave the cassettes for sterilization, even if only media is dispensed. Any proteinaceous liquid is subject to clogging when alcohol is used. Dedicating a cassette to each cell line is highly recommended.

**Protocol Optimization – Selecting the Right Speed**
The dispensing parameters need to be optimized for each cell line. Parameters depend on the cells and suspension materials being used. When dispensing cells with the Multidrop Combi, there are three speeds which you can choose from; low, medium, fast. Typically a low to medium speed would be best for cells and media due to frothing of suspension. For some cell lines and concentrations, a slow speed might block the tips or if the speed is too fast the cell suspension might have foam in the wells. In some cases, small droplets may be formed on the ends of the tips with slow speed. Increasing the speed is needed to eliminate this phenomenon.
Cleaning Guidelines After Dispensing Cells

Procedure to clean clogged tips:
1. Press EMPTY button for a few seconds. Then press the PRIME button for a few seconds.
2. Place a reservoir filled with a mild detergent solution under the cassette tips so that the tips are submerged in liquid and press the EMPTY button.
3. Prime with ddH₂O.

Procedure for disinfection:
1. Clean dispensing cassette thoroughly with mild detergent and ddH₂O.
2. Prime with ddH₂O.
3. Prime with 2% Virkon liquid for disinfection purposes. Adequate priming is achieved when the priming vessel is full. Let Virkon remain in the dispensing cassette for about 15 minutes.
4. Prime tubings again with ddH₂O.

It is not recommended to use bleach for cleaning the dispensing cassettes as it may cause reactions with the reagents, cells or other sensitive substances e.g with the microplate used. Even if the bleach is rinsed very carefully with ddH₂O from the dispensing cassette, the effectiveness of the bleach being completely rinsed away has not been tested.

Additional Tips and Tricks
- Standard cassettes are optimized for dispensing volumes over 50µl, but can be used for any volume greater than 20µl.
- Prime tubing with PBS to wet the tubes before dispensing cells. For the small tube cassette, run approximately 2ml through the tubing; for the standard tube cassette, approximately 10ml.
- After PBS, prime cassettes with the cell suspension to ensure even distribution including the first few columns. For small tube cassettes, this would be approximately 1.5ml and for the standard cassette, approximately 8ml. If the priming is inadequate, the first columns may not have the same amount of cells as the last columns dispensed due to cells tendency to attach to silicone.
- An orbital shaker is recommended to keep cells gently in motion while dispensing. This not only helps prevent clumping, it also allows the Multidrop Combi to dispense a more consistent concentration of cells across the plate.
- After dispensing, flush tubing with 10-15ml PBS. You can leave PBS in the tubing for up to 1 hour. After longer pause, flush the tubing with ddH₂O and empty them to prevent salt or protein clogs in the tips.
- When dispensing is completed for the day, clean the cassette by priming with a mild detergent such as TWEEN-20 or Triton X-100 or with a cleaning solution such as 1% Micro-90. For small tube cassettes, run approximately 10ml through the tubing; for the standard tube cassettes, run approximately 20ml through the tubing.
- After using the detergent, prime tubings with ddH₂O (10ml for small cassettes, 20ml for standard cassettes) and repeat with a new vessel of ddH₂O to prevent detergent carry-over.
- Avoid using ethanol to dry the tubes after dispensing cells or protein solutions! Ethanol precipitates proteins, which can lead to clogged tips. If you wish to sterilize the cassette, autoclave it.
Additional Cell Dispensing – Cleaning Instructions
For additional detailed information on proper dispensing of cells and cleaning guidelines for cassettes:

• TN-ALH-MDcombi01-0209: Maintenance Guide for Cell Dispensing with Thermo Scientific Multidrop Combi

• TN-ALH-MDcombi02-0709: Reproducible Dispensing of Live Cells with the Thermo Scientific Multidrop Reagent Dispenser

Cleaning Unit / Decontamination
When working with cells, it is good laboratory practice to periodically clean the outside of the instrument with a cloth dampened with water, 70% ethanol or a mild detergent. Refer to detailed decontamination instructions from the Multidrop Combi User Manual.

Summary
When using the Multidrop Combi dispensers to dispense cells, it is recommended to properly prepare and clean the cassettes to maintain the viability and provide accurate and reproducible performance until the cassettes are at end of life (EOL). Following these guidelines will help to ensure the cassette remains properly cared for by the user.