

Maintenance guide for cell dispensing with Thermo Scientific Multidrop Combi



The peristaltic pump technology of the Thermo Scientific Multidrop Combi and Multidrop dispensing cassettes assure a fast and gentle way of dispensing cells on any plate and well size. Cells remain intact and viable, and the plate can be used for any cell-based assay needed. This technical note describes practical guidelines for cell dispensing with Multidrop Combi and dispensing cassette maintenance.

Selection of the dispensing cassette

Selection of the optimal dispensing cassette for cell suspension depends on the volume of the dispensing and the plate type (i.e. the size of the well).

Volume

- Small tube dispensing cassette: Dispensing volume < 50 μl (plastic tips recommended rather than metal tips)
- Standard tube dispensing cassette: Dispensing volume > 50 μl

Plate type

- Small tube dispensing cassette: 96-, 384- and 1536-well plate
- Standard tube dispensing cassette: 6-, 24-, 48-, 96- and 384-well plate

It is highly recommended to use a separate dispensing cassette for different cell lines in order to avoid any contamination or mixing of the cell lines.

Priming the dispensing cassette

Before dispensing the cell suspension, prime the instrument with PBS to wet the tubing and tips. The volume of the PBS for priming depends on the dispensing cassette.

- Small tube dispensing cassette: ~2 ml (including dead volume for filling the tubing)
- Standard tube dispensing cassette: ~10 ml (including dead volume for filling the tubing)

Prime the dispensing cassette with the cell suspension before dosing to reach an equal distribution of the cells on the plate. The volume of the cell suspension needed for priming depends on the dispensing cassette.

- Small tube dispensing cassette: dead volume + 500 μl
- Standard tube dispensing cassette: dead volume + 1 ml

Dispensing

The maximum cell concentration for the dispensing depends on the cell line. It is possible to spread the cells evenly in the well by dispensing cells with the Multidrop[®] Combi. Figure 1 shows the distribution of the cells on the 96-well plate with two cell concentrations.

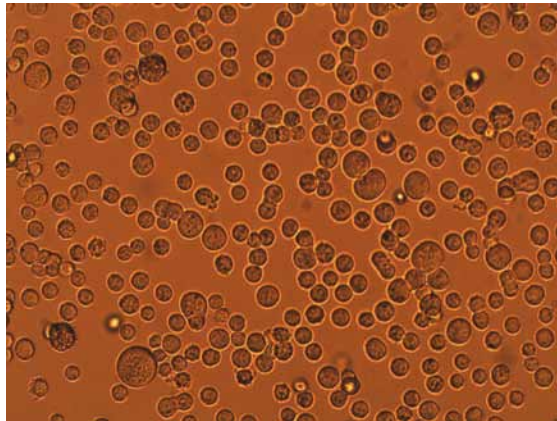
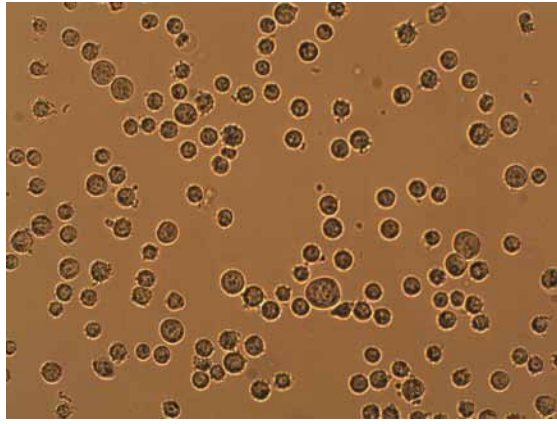


Figure 1. Dispensing of 50 μ l of HeLa-S3 cells with the small tube plastic dispensing cassette onto the 96-well plate. a) Cell concentration 2.9×10^5 cells/ml. b) Cell concentration 6.5×10^5 cells/ml

Practical tips on cell dispensing

- When dispensing several plates, use for example, the orbital shaker for gentle mixing while dispensing to keep the cells suspended.
- A stir plate with magnetic stir bar in the cell suspension is effective for maintaining the cells in suspension. The cells should be stirred at a fairly slow speed, just enough to keep them in suspension, but not enough to create a vortex or frothing, which will adversely affect cell viability. Ensure that the tubing stays to one side, out of the way of the stir bar.
- When dispensing the same cell line later in the same day, prime with PBS to rinse the tubing after the dispensing and before starting a new dispensing later. It is possible to leave the tubing filled with PBS for 1 hour. If the time between dispenses is longer, rinse the tubing with ddH₂O to avoid any precipitation on the tips.

Cleaning the dispensing cassette

Clean the dispensing cassette shortly after dispensing to avoid any clogging!

After dispensing cells, rinse the tubing by priming with 10-15 ml PBS to remove cells remaining on the tubing. To fully clean the tubing and the tips, complete the following washing instructions. Separate dispensing cassettes for different cell lines recommended to ensure that the cell lines do not contaminate.

To clean the dispensing cassette, prime with a mild detergent (e.g. TWEEN[®]-20 or Triton[™] X-100) or a cleaning solution (e.g. 1% Micro-90[®] Cleaning Solution by Cole-Parmer) and to disinfect tubing, rinse with 10% bleach.

- Small tube dispensing cassette: 5-10 ml
- Standard tube dispensing cassette: 15-20 ml

After cleaning the tubing, prime twice with ddH₂O to rinse the tubing. Use two separate tubes of water. There are remains of the detergent on the outer surface of the tubing that mixes with the first rinsing water.

- Standard tube dispensing cassette : min. 20 ml
- Small tube dispensing cassette: min. 10 ml

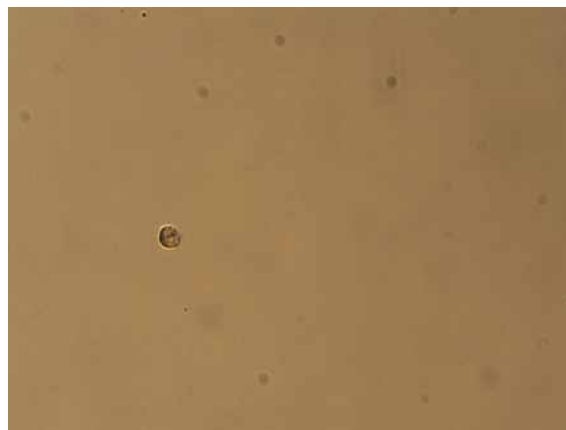
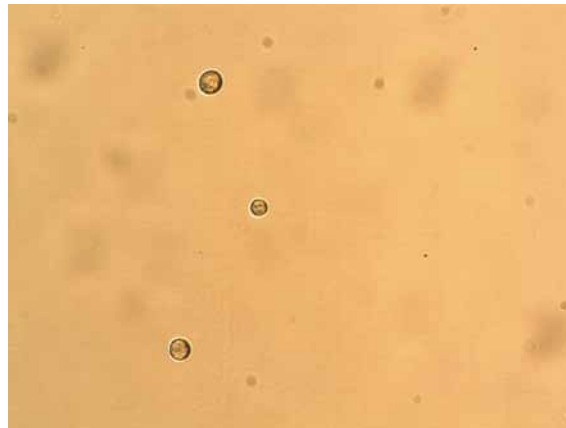


Figure 2. Rinsing the tubing with PBS. a) after priming with 5 ml of PBS, and b) after priming with 10 ml of PBS.

Troubleshooting

Clogging

Reasons for clogging:

- Cell clumps in the suspension
- Leaving the cell culture to stand in the tubing
- No priming with PBS done to wet the tubing
- Ethanol used for washing the tubing after cell dispensing (precipitates proteins)

To clean the blockage:

- Use a mild detergent or cleaning solution (it is possible to let the detergent remain in the tubing for 5 to 10 minutes to break down the cells and dissolve the clog)
- Prime and empty the tubing several times with a mild detergent and rinse properly with ddH₂O
- Using the enclosed syringe and tubing to backflush clogged tips individually is usually effective. Always remember to rinse with ddH₂O.

To prevent clogging:

- Use an even suspension of single cells
- Prime with PBS before and after dispensing
- Clean the dispensing cassette immediately after dispensing

Frothing

The cell culture media includes serum, that may cause frothing, especially on the metal tip cassette.

Tips on how to minimize frothing:

- Do not empty the tubing between dispensing different liquids, and use prime to replace the solution with the new liquid
- Use a lower dispensing speed, medium speed usually works best
- Use a lower mixing speed
- Use less serum, if possible, or switch to a low concentration of BSA (0.1% or 0.05%) if cells do not need to be attached

Uneven amount of cells per well

Insufficient priming with the cell suspension:

- Follow the instructions for priming the dispensing cassette

The cell tube/bottle has been standing without gentle mixing before dispensing:

- Create the dispensing protocol beforehand
- Have the right amount of plates in hand (next to the instrument)
- Gently mix when dispensing several plates

A clumpy cell suspension:

- Check the cell suspension for clumps

Unexpected amount of dead cells after dispensing

Check your cell batch viability before dispensing.

Insufficient rinsing can affect the cell viability:

- Rinse tubing thoroughly after cleaning with bleach or mild detergents

Water can affect the cell viability:

- If the tubing has been wet with water, prime with PBS before dispensing

Further Information

For further information about Multidrop Combi, please refer to the following web pages:

- www.thermo.com/multidrop
- www.thermo.com/mpi

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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