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I-Dot One Protocol

Dispensing Cells

This is a suggested procedure, please adjust according to your experimental needs.

Protocol aim

The aim of this protocol is to provide instructions to accurately and reproducibly dispense cells on Droplet Microarray (DMA) slides with the I-DOT One. Cells will be suspended in their appropriate medium.

Material needed

- I-DOT One*
- Pure Plate 90 µm*
- Droplet Microarray (DMA) **
- DMA-Adaptor**
- Humidifying Pads
- Humidifying Buffer
- Cells in suspension
- Culture medium
- 10 mL Conical Centrifuge tubes
- Sterile tweezers

* The product can be purchased in the CELLINK shop at https://cellink.com/biosystems/.
** Droplet Microarrays can be purchased from Aquarray http://www.aquarray.com .
Deposit according to local regulations.

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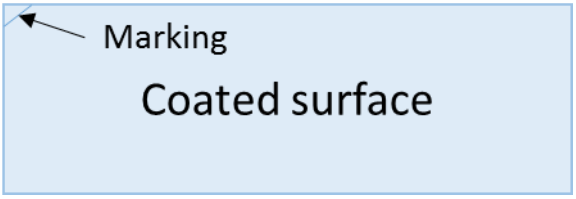
Protocol

This protocol is described for dispensing cell suspensions with the I-DOT One.

Step	Title	Material	Description
1	Create protocols on I-DOT	- I-DOT One	- Select appropriate DMA format in the menu. - For providing and installing DMA formats please contact CellInk - Select target wells locations, select the liquid, and set the dispensing volume at 150 nL for each spot on the DMA. - Select source well locations. Note: The liquid and liquid class needs to be calibrated. Refer to *I-DOT One Manual* for procedure and in-depth procedures for setting up I-DOT One protocols.
2	Sterilize DMA	- DMA	- DMA slides are absolutely transparent with top surface coated and bottom surface non-coated. **The coated surface should not be touched** with gloves of tweezers **to avoid the damage of the coating**. Before taking the slide out of the falcon tube, examine the slide and find the coated surface by a marking at upper left corner (see the figure below). The DMA can be held with gloves on the sides or with tweezers at the very corner. Droplet Microarray can be placed in Petri dish or any other container on the non-coated surface.  - Insert one transparent DMA into 50 mL falcon tube containing 45 mL of 70 % ethanol for 10 minutes with the mark to

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			the top (do not incubate in ethanol longer than 10 minutes). Remove transparent DMA slide from ethanol under clean bench and place them **on non-coated surface** onto the edge of open sterile Petri dish to dry at least for 15 minutes.
3	Preparation of Petri dishes with humidified lids	- Petri dish (one per DMA) - humidifying pad - humidifying buffer	- Humidifying pads should be autoclaved in appropriate container before use. Humidifying buffer should be autoclaved in an appropriate container or sterile filtered before use. - Open the lid of the Petri dish and place it upside down under clean bench. - Place a humidifying pad into the lid using sterile tweezers. - Add 5-10 mL of sterile humidifying buffer to the lid and let it evenly spread over the whole surface of the pad. Important: the pad has to be fully wetted; extra liquid might cause detachment of the pad and has to be collected with a sterile pipet from the edge of the lid. - Add 2 mL of the humidifying buffer inside the Petri dish next close to the middle. If some air is trapped between the pad and the lid squeeze it out with a tip of the pipette
4	Create cell suspension	- Cells in suspension - Culture medium - 10 mL Centrifuge tube	Create cell suspension with $1.5x10^6$ cells in 1 mL to reach 100 cells/ 150 nL micro droplet (to achieve different number of cells per spot, adjust the cell concentration accordingly): - Trypsinize cells following standard procedure, transfer them in 15 mL centrifuge tube and centrifuge for 3 minutes at 1200 rpm. - Aspirate the supernatant. - Resuspend the cell pellet in 1-2 mL of seeding medium. - Count the cells.

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			- Dilute the cells till final concentration of $1.5x10^6$ cells/mL (to obtain 100 cells per droplet) with seeding medium.
5	Set-up Humidity Control		- Switch on humidity control of the I-DOT 10 minutes before the cell seeding. Set up humidity control for 70%. Humidity control is essential for dispensing cells in nanoliter volumes on DMA. To upgrade your I-DOT One with humidifier, please contact Celllink .
6	Load DMA	- I-DOT One - Droplet Microarray	- Place the DMA slide **on its non-coated surface** into a DMA-adaptor (DMA - adaptor can be purchased from Aquarray) using sterile tweezers. Make sure that the DMA slide is fully inside the frame of the adaptor. Then slightly push the DMA slide to the left side of the adaptor with a sterile tweezer. Close the tray. - Load the DMA with the DMA adapter in the lower tray of the I-DOT.
7	Load cell suspension	- I-DOT One - Pure Plate 90 µm - Cell suspension	- Load the Pure Plate 90 µm plate into the source tray. - Place required amount (count 5-10 µL more than calculated for the total amount for dispensing) of cell suspension into a well of a source plate, mix well!
8	Dispense and transfer	- I-DOT One	- Select “Start dispensing” in the “Dispense” tab on the I-DOT One. - Bring prepared the Petri dish with humidifying pad next to the I-DOT dispenser. After dispensing is completed open the tray with the DMA slide and transfer the DMA with sterile tweezer immediately into the Petri dish with humidifying pad. As a rule, when handling DMA containing cells, do not open the Petri dish lid completely, but open it just enough for placing or removing the DMA slide. Place the

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			Petri dish with DMA slide inside a standard cell culture incubator.

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