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

Protocol aim

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

Preparation of staining solution:

Hoechst 33343: ThermoScientific, #62248; final dilution 1:10000 (excitation 355nm laser)
Propidium Iodide: ThermoScientific # BMS500PI; final dilution 1:3000 (excitation 535nm laser)
Calcein: ThermoScientific #C1430; final dilution 1:2000 (excitation 945 nm laser)

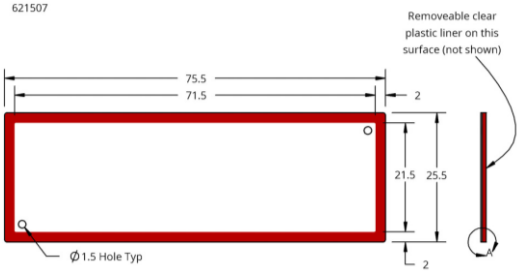
For one 672 DMA 33,6 µL of staining solution (exact calculation) would be required for each slide.

Prepared dilutions have to be 4x more concentrated as final dilution:
Hoechst 1:2500
PI 1:750
Calcein 1:500

Material:
DMA with 672 spots
SecureSeal™ Hybridization Chamber (21,5x71,5x0,8 mm): Grace Bio-Labs (#621507)

Preparing a final volume 200 µL:

1. Add 0,08 µl Hoechst and 0,26 µL Propidium Iodide to 199 µL PBS.
2. Print 50 nL of staining solution on each droplet (150 nL culture medium containing cells) with protocol “staining with Hoechst-Calcein-PI on 672 DMA”.
3. After addition of the staining solution put the DMA back the into Petri dish with humidified lids and incubate for 15 min in the cell incubator at 37°C.
4. Meanwhile prepare a SecureSeal chamber



(image taken from Grace Biolabs)

5. Cut two small squares of a transparent adhesive tape to close the two holes (Ø 1,5mm) on the left lower and right upper part.
6. Remove the DMA carefully from the Petri dish with humidified lids and put the sticky chamber quickly on the DMA to avoid drying of the cells/droplets. Ensure that the sticky chamber is well fixed on the DMA. Transfer to the microscope for imaging.