Flow Post-its
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HRP Verification for Single Cell Sorting into Multi-well Plates

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Cell sorters provide a fast and accurate method to isolate cells. When carrying out **single cell sorting** into **plates,** it is important to **ensure successful droplet deposition** into the fluid at the bottom of the well. Here we review a rapid and inexpensive **colorimetric method** developed by Rodrigues et al. (2016) to verify whether only a single droplet was deposited in each well during plate sorting.

Supplies Needed

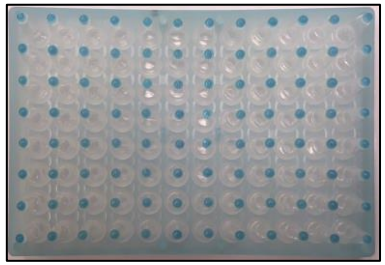
1. TMB ELISA Substrate Solution
2. Horseradish Peroxidase (HRP)
3. Flow-Check Fluorosphere beads
4. 1 X Phosphate buffered saline (PBS)
5. Multichannel pipette
6. 96 or 384 well plate
7. Disposable reagent reservoir

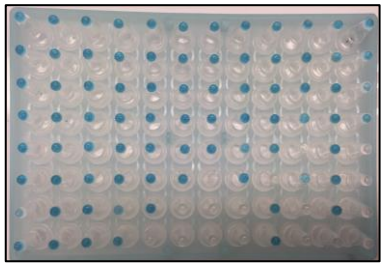
Procedure

1. Pre-warm TMB at room temperature for 30 minutes.
2. Dilute 5 mg/ml HRP stock to 250 µg/ml working solution with 1XPBS
3. Add several drops Flow-Check beads to 500 µl HRP working solution.
4. Transfer TMB solution into empty plate with a multichannel pipette. The volume should be same as the volume of buffer used in the experiment.
5. Load the beads prepared in step 3 onto the sorter and set scatter gate around the population of the beads.
6. Select all wells on the plate layout for sorting. Add scatter gate as the sorting population and set the target number 1 for each well. Ensure you are in **single cell** sorting mode.
7. Start acquiring the beads and sort.

Color Development

Centrifuge the plate at 2000 g for 2 minutes and let the color develop. Keep plate protected from light during incubation. Inspect the plates and proceed sorting your sample or recalibrate as necessary.





Good result: All wells uniform shade of blue. No empty wells.

Bad result: Multiple wells did not have droplet deposition.

Helpful Hints

- HRP can reduce H_2O_2 to O_2 and H_2O by using TMB as the donor molecule. TMB is colorless in its reduced substrate form. It turns blue when it is oxidized.
- Use TMB as quick as possible once it is aliquoted. Exposure to normal atmospheric conditions can turn TMB to blue even without substrate.
- Lack of color change indicates a droplet did not make it to the well in question.
- A darker shade of blue suggests that more than one droplet was sorted into the darker well.

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