

Video Article

CD4⁺ T-Lymphocyte Capture Using a Disposable Microfluidic Chip for HIV

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Abstract

Video Link

The video component of this article can be found at <http://www.jove.com/video/315/>

Protocol

1. Prepare microfluidic channel with anti-CD4⁺ biotinylated surface.
2. Inject 10 ml of sample blood at 5 ml/min into the microfluidic channel with syringe pump.
3. Flush out the blood at 20 ml/min for 20 min with PBS.
4. Inject diluted FACS lysing solution (diluted to 10% from stock solution with PBS) at 5ul/min for 15 min.
5. Incubate for 15 min.
6. Inject primary CD3⁺ antibody solution (diluted to 10% from stock solution with PBS) at 5 ml/min for 20 min.
7. Incubate for 2 hours.
8. Flush out channel with PBS at 5 ml/min for 15 min.
9. Inject 20% AF488 CD4⁺ antibody, 0.2% DAPI stock solution, and 10% secondary CD3⁺ antibody at 5 ml/min for 20 min at the same time.
10. Incubate for 20 min.
11. Flush out the channel with PBS at 5 ml/min for 15 min.
12. Count cells under fluorescent microscope:
 1. White (optical) - Black and white: cell and dust
 2. UV(DAPI) - Blue: cell only
 3. Blue(CD4⁺) - Green: T-lymphocyte and monocyte
 4. Green(CD3⁺) - Red: T-lymphocyte only