Introduction

CD137 has been reported to be a marker for antigen (e.g. peptide)-specific activation of human $CD8^+$ T-cells. It is undetectable on unstimulated $CD8^+$ T-cells, but uniformly upregulated approximately 24h after peptide stimulation on virtually all responding cells, regardless of differentiation stage or cytokine secretion profile. Antibody-labeled responding $CD137^+$ cells can be easily and efficiently isolated using a FACS machine or magnetic beads to substantially enrich antigen-specific T cell populations.

Materials and Equipment

- Cell sample, e.g. Blood sample (RBC-depleted), PBMCs or T cell line
- Anti-CD137 antibody conjugated to the fluorescent label of choice (e.g. APC)
- Anti-fluorochrome magnetic beads, e.g. anti-APC (Miltenyi Biotec / BD Biosciences), to detect the fluorescence of the anti-CD137 antibody
- Anti-CD8 antibody, conjugated with a different fluorescent label to the anti-CD137 antibody
- Wash buffer (0.1% sodium azide, 0.1% BSA in PBS) – remove air prior to use (de-gas)
- Fix solution (1% fetal calf serum, 2.5% formaldehyde in PBS)
- Magnetic stand e.g. MACS Multistand, Miltenyi Biotec #130-042-303
- Separation unit e.g. MiniMACS separation unit, Miltenyi Biotec #130-042-102
- Magnetic columns e.g. MS columns, Miltenyi Biotec #130-042-201
- Benchtop refrigerated centrifuge with swing-out rotor and appropriate carriers

Procedure for washing cells

Dispense 1 ml wash buffer per tube and spin 400 x *g* for 5 minutes in a chilled centrifuge at 4ºC. Check for presence of a cell pellet before discarding the supernatant. Resuspend cell pellets in residual liquid (~50 µl).

Staining Protocol

1. **Allocate 1-2 ´ 10^6 lymphoid cells (PBMC or splenocytes) per staining condition.**
2. **Wash cells with wash buffer and resuspend in the residual volume (~ 50µl).** Keep tubes chilled on ice for all subsequent steps, except where otherwise indicated.
3. **Add an optimal amount of anti-CD8 and anti-CD137 antibodies (and any other desired antibodies) and mix by pipetting.** If staining control samples with other primary antibodies, at this stage add an optimal amount to the cells in their respective tubes.
4. **Incubate samples on ice for 20 minutes, shielded from light.**
5. **Wash cells twice with 2ml wash buffer per tube. Mix each tube.**
6. **If analyzing by flow cytometry, add 200µl fix solution. Vortex tubes.** It is important to vortex well when adding fixative so that cells do not clump. Store tubes in the dark in the refrigerator until ready for data acquisition. The morphology of the cell changes after fixing, so it is advisable to leave the samples for 3 hours before proceeding with data acquisition. Samples can be stored for up to 2 days.

   If sorting the cells in a FACS machine, add 1ml wash buffer containing a DNAse, such as Benzonase. Vortex tubes. The DNAse prevents cell clumping during the sorting process. Proceed with sorting the cells as soon as is possible.

Bead-isolation Protocol (may need adjustment depending on the particular system used)

1. **Stain 1×10^7 cells with an optimal amount of anti-CD137 antibody for 20 minutes at 4ºC.**
2. **Wash samples, centrifuge and discard the supernatant.**
3. **Resuspend cells in 80µl wash buffer and add 20µl anti-fluorochrome magnetic beads per tube** (or as directed by the manufacturer).
4. **Incubate for 15 minutes at manufacturer's recommended temperature.**
5. **Wash cell-bead complexes, centrifuge and resuspend in 500µl wash buffer (de-gassed).**
6. **Meanwhile, wash a column suitable for positive-selection with 500µl wash buffer (de-gassed) and place on magnetic stand.**
7. **Load cell-bead complexes onto the column.** Antigen-specific T cells (labeled with anti-CD137-bead complexes) will be retained on the column (positive fraction).
8. **Collect the negative fraction that elutes from the column, including 3 column washes of 500µl each.**
9. **Remove column from magnet and flush out the positive fraction by adding 1ml wash buffer onto the column and applying a plunger (provided with the column).**
10. **To obtain a purer antigen-specific cell population, the positive cell fraction may be passed over a second column.**
11. **Stain cells from pre-isolation, positive and negative fractions with anti-CD8 antibody for flow cytometric analysis.**

References

Wölfl, et al. (2007). Activation-induced expression of CD137 permits detection, isolation and expansion of the full repertoire of CD8+ T-cells responding to antigen without requiring knowledge of epitope-specificities. Blood 110: 201-210.

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