T cell activation assay

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Abstract

This protocol describes a method for detecting T cell activation in response to BiTE® treatment by flow cytometry .

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Protocol

Overview

Step 1.

Supernatants (containing T cells) are removed from TDCC assays (please refer to TDCC protocol). In a multiplexed flow cytometry assay, cells are stained with anti-CD45 (lymphocyte common antigen) and antibodies to the T cell activation markers CD25 and CD69. CD45 positivity distinguishes T cells from cell debris. Analysis identifies the populations that are $CD45^+$ and $CD69^+$ and/or $CD25^+$.

Assemble materials

Step 2.

Materials	Company	Cat.no.
Sterile 96-well clear V-bottom polypropylene plates	Greiner	651201
1x PBS	Gibco	14190
Heat-inactivated fetal bovine serum (FBS)	Gibco	10082-147
Mouse anti-human CD45-APC	BD Biosciences	555485
Mouse anti-human CD69-FITC	BD Biosciences	555530
Mouse anti-human CD25-PE	BD Biosciences	341009

Prepare reagents

Step 3.

- FACS buffer (cold PBS + 2% FBS)
- Prepare master mix: 25 µl PBS + 250 µl each mouse anti-human CD45-APC, CD69-FITC and CD25-PE

Prepare T cells for flow cytometry

Step 4.

- Resuspend pelleted T cells in 100 µl cold FACS buffer (PBS + 2% FBS)
- Centrifuge at 300 x g for 10 min at 4°C
- Remove supernatants with vacuum manifold or pipet

- Add 100 µl cold FACS buffer/well
- Centrifuge at 300 x g for 10 min at 4°C
- Remove supernatant with a vacuum manifold or a pipet and resuspend cells in 60 µl cold FACS buffer.
- Add 30 µl cold master mix to each well
- Mix and incubate for 30 min at 4°C
- Wash 2 times with 100 µl cold FACS buffer, as above
- Resuspend cells in 100 µl cold FACS buffer
- Acquire data on FACS CANTO