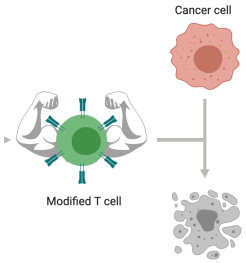
Oct 18, 2019 Version 1

Flow-cytometry-based in vitro assay for assessing T-cell-mediated cytotoxicity against a target cell line (24-well plate, pmel-1 or OT-I T cells, MC38 cell line) V.1



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Protocol status: Working
We use this protocol and it's working

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Keywords: T cells, cytotoxicity, co-culture, MC38, TCR, CAR, flow cytometry, CD3, CD8, mediated cytotoxicity, cytometry, amount of cell, number of target cell, target cell, alternative to these release assay, mc38 cell line, release assay, important assay, target cell line, cell, cell line, type of cell line, assay

Abstract

In vitro co-cultures of cytotoxic T cells with their target cells are important assays to asses the functionality of the T cells in a scalable way. These assays rely on co-culturing CD8 T-cells, often times genetically modified to express a specific TCR or CAR, with another type of cell line that can be recognized by T cells. Co-cultures are typically run for 6-24 hours and then the amount of cells that were killed in the co-culture can be assesed through different techniques -- e.g. radioactive Cr or non-radioactive LDH release assays. Here, we outline another alternative to these release assays which relies on flow cytometry to estimate the number of target cells left in the culture after a certain period of time.

Materials

MATERIALS

- Trypsin 0.05% 1X Solution **VWR International (Avantor)** **Catalog #**16777-202
- CytoOne 24-well TC plate **USA Scientific** **Catalog #**CC7682-7524
- APC anti-mouse CD3 Antibody **BioLegend** **Catalog #**100235
- PerCP anti-mouse CD8a Antibody **BioLegend** **Catalog #**100731
- pmel-1 mouse (B6.Cg-Thy1a/Cy Tg(TcraTcrb)8Rest/J) **The Jackson Laboratory** **Catalog #**005023
- OT-I mouse (C57BL/6-Tg(TcraTcrb)1100Mjb/J) **The Jackson Laboratory** **Catalog #**003831

STEP MATERIALS

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Troubleshooting

Before start

- Make sure you have enough activated (for at least 3 days), healthy (>50% viability), and cytotoxic (CD8) T cells in culture before starting
- Make sure you have access to a flow-cytometer after the co-culture is done
- When in doubt, use 24-well plates for the co-culture
- Make sure the cell line expresses the target protein (for CAR) or presents the relevant peptide (for TCRs) up-front
- Make sure the cell line can grow and sustain viability in T cell media throughout the co-culture
- Make sure the final T cell concentration doesn't go higher than 2 million per mL since this can cause stress on the T cells and the cell line
- This protocol assumes the assay is carried out at 8:1 T-cell:Cell-line ratio. Please scale the numbers up if you would like to assay at a different scale/ratio
- When in doubt, use OT-I CD8 T cells against MC38s that are pulsed with the SIINFEKL peptide as a positive control
- When in doubt, use OT-I CD8 T cells against MC38s that are NOT pulsed with the SIINFEKL peptide as a negative control
- This protocol assumes the T cells and the cancer cells are of mouse origin. If you are using a different organism or the channels are not appropriate for your flow-cytometer, please customize your antibodies accordingly

Day 0 - Seeding the target cells

1 Collect at least 3 million MC38s by trypsinizing them from an on-going culture

2 Spin them down at 200 x g for 00:05:00 at 4 °C and re-suspend them in fresh media at a 250,000 cells per mL concentration

3 Seed each 24-well-plate well with 500 uL of the cell suspension (i.e. 125,000 MC38 cells per plate)

4 Incubate overnight and allow cells to adhere to the plate

Day 1 - Co-culture

5 Collect 2 million cytotoxic T cells per sample (i.e. per well) from an on-going culture

6 Spin them down at 350 x g for 00:05:00 at 4 °C and re-suspend them in fresh media at 1 million per mL concentration

7 Supplement T cells with 200 IU/mL rIL2

8 Aspirate the culture media from each of the 24-well-plate wells that contain a sample. Try to aspirate as much as possible but make sure you don't disturb the adherent cells during this process

9 Add 2 mL of the T cell suspension onto each of the sample wells. Assuming that the cancer cell line doubled overnight, this would result in a 8:1 (2 million:250K) T-cell:MC38 ratio.

10 For positive controls (samples that are expected to get killed), if the cancer cell line doesn't express or present the target protein/epitope, make sure to supplement the co-culture with the target peptide.