51Cr Release Cytotoxicity Assay for murine CAR T cells

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AUG 21, 2023

ABSTRACT

A standard cell-based assay to assess the function of murine CAR T cells, which we regularly perform at the end of the process of generating CAR T cells (see "Retroviral transduction of primary murine CD8 T cells").

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldmm2ol5b/v1

Protocol Citation: Tamer B Shabaneh, Andrew R Stevens 2023. 51Cr Release Cytotoxicity Assay for murine CAR T cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldmm2ol5b/v1

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

Created: Aug 20, 2023

Last Modified: Aug 21, 2023

PROTOCOL integer ID: 86711

Labeling target cells with 51Cr (day 0)

1 For each adherent tumor cell line, plate 5x10^5 cells / 3mL (previously growing in log phase) in one 6-well-plate well; Incubate cells for ~3hr to rest.

2 **In the Hot Lab**, label the target cells with 50 µL ^{51}Cr (vial <1wk old), 100µL (1-2wk old), or 150µL (>2wk old). Place in Hot Lab incubator overnight.

Note

Overnight chromium incorporation is often sufficient, but the duration may vary depending on the cell line. If the positive control (soap-killed cells) endpoint values are inadequate, the length of time can be adjusted.

Processing effector cells (day 1)

3 From each transduced T cell culture, remove 9x10^5 cells, wash with fresh mouse T cell media (mTCM) for 6min at 400rcf, and resuspend in 3mL mTCM.

Note

mTCM is prepared by combining the following:

1000 mL	RPMI1640 (with 25mM HEPES)
100 mL	FBS (heat inactivated)
10 mL	Sodium pyruvate (1mM)
1 mL	HEPES (1M)
10 mL	Pen/Strep
100 mL	b-mercaptoethanol 0.5M

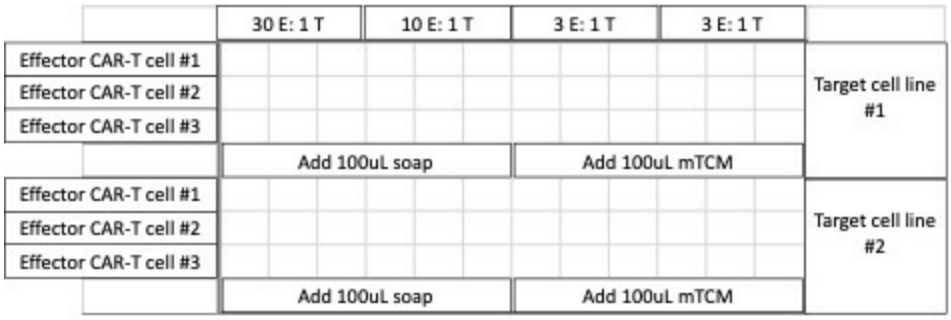
Filter with 0.22mm

4 In triplicates, transfer 150µL (4.5x10^4 effector cells) to U-bottom 96-well-plate wells (in columns 1, 2, 3). Pipet mTCM (100uL/wel) into the remainder of the row (4-12); prepare 3 serial dilutions (1:3) by pipetting 50uL to the respective 100uL volumes.

Note

This would assess cell killing for one CAR T cell culture against one target. Scale up as needed by seeding additional rows.

4.1 Example layout:



Desired E:T ratios are 30:1 [$3x10^4$:$1x10^3$], 10:1, ~3:1, and ~1:1

5 **Begin processing the target cells.** To remove extracellular 51Cr, briefly wash the adherent lines with 5mL Cell Dissociation Buffer (0.5mM EDTA in PBS). Repeat the wash. Dispose the 51Cr-containing media appropriately.

Note

Non-enzymatic dissociation is a critical step for preserving cell-surface antigen.

5.1 To dissociate target cells, add 3mL Cell Dissociation Buffer to each well, incubate at 37C for 10-15 mins. Add 3mL mTCM; centrifuge at 400rcf for 4min.

6 Count the cells using a hemocytometer. Adjust volume to **1E4 cells / mL** mTCM. Transfer **100µL** (1E3 target cells) to each well on the respective half of the plate already containing 100uL of effector CAR-T cells.

7 Finally, transfer 100µL target cells to an extra row. Add 100µL of soap (NP40+Trypan blue) to

generate a positive control. Add 100µL mTCM to generate a negative control.

8 Centrifuge plate at 800rpm for 3mins; incubate at 37C for 24 hours.

Reading scintillation plate

9 Harvest 30uL supernatant from each plate; carefully transfer onto respective Luma plate wells. Dry the plate completely for ~24 hours.

10 Acquire data on the plate scintillation counter per respective protocol.