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51Cr Release Cytotoxicity Assay for murine CAR T cells

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We use this protocol and it's working

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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").

Labeling target cells with ^{51}Cr (day 0)

- 1 For each adherent tumor cell line, plate 5×10^5 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), 100 μL (1-2wk old), or 150 μL (>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 9×10^5 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 150 μL (4.5×10^4 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:

	30 E: 1 T	10 E: 1 T	3 E: 1 T	3 E: 1 T	
Effector CAR-T cell #1					Target cell line #1
Effector CAR-T cell #2					
Effector CAR-T cell #3					
	Add 100uL soap		Add 100uL mTCM		
Effector CAR-T cell #1					Target cell line #2
Effector CAR-T cell #2					
Effector CAR-T cell #3					
	Add 100uL soap		Add 100uL mTCM		

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

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

