Murine CD8 T cell restimulation in vitro

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ABSTRACT

This protocol outlines the steps for re-stimulating engineered murine CD8 T cells in vitro.

In brief: Transduced T cells were re-stimulated with irradiated Thy1.2+ splenocytes pulsed with $Msln_{404-414}$ peptide (GQKMNAQAI, 1ug/mL) and IL-2 (50 IU/ml) for 5 days following T cell activation with a-CD3e/CD28. Peptides were purchased from Elim Peptide at >80% purity. Three days after the second transduction, >40% (TCR_{OTI}) and >85% (TCR_{1045}) of T cells expressed the transduced TCR or construct. On day 7 after antigen re-stimulation, >90% of T cells expressed the transduced TCR.

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MATERIALS TEXT

MATERIALS

Falcon 50 mL polypropylene conical tube **Corning Catalog #352070**

15 mL polypropylene centrifuge tube **Corning Catalog #430791**

Falcon cell strainer, 70um, nylon **Corning Catalog #352350**

BEFORE STARTING

You will need access to an irradiator to prepare irradiated antigen-presenting cells for this protocol. Access to this equipment often requires a federal background check. Reach out to your institution to determine appropriate requirements to access this equipment.

Day 0: Prepare peptide-pulsed antigen-presenting cells

1 **1.** Obtain spleens to pulse with peptide. Record pertinent information regarding donor mice (mouse background, number of spleens, gender, date of birth).

2. Harvest T cells by smashing spleens through a 40um filter. Rinse with 10-20 mL of T cell media and transfer to a 15 or 50 mL tube. Spin down @369*xg* for 4-5 min at 4C.

3. ACK Lysis with 2 ml/spleen for 2 min at room temperature.

4. Quench ACK lysis with T cell media (use an equal or greater amount than the ACK volume).

5. Spin down @369*xg* for 4-5 min at 4C. Resuspend in T cell media and split cells into separate 15 mL tubes to irradiate (1 tube per peptide condition; aliquot 1ml of cells / tube.).

6. Irradiate at 3500 rad (35 Gy).

7. Rinse cells immediately by filling tube with 10-20 mL of T cell media. Spin down at @369*xg* for 4-5 min at 4C. Resuspend in 1-2 mL T cell media. The volume will depend on the number of spleens/cells in the tube. The goal is to pulse with excess peptide. Typically 1ug of peptide is sufficient to pulse up to 5 spleens worth of cells.

8. Add appropriate amount of peptide per tube. (e.g. 1ug of peptide for 1-5 spleens)

9. Mix well and incubate at 37C for 60 to 90 minutes (can go longer if necessary).

10. Rinse excess peptide out by washing with 10-12 mL T cell media.

11. Rinse a second time with 10-12 mL T cell media.

12. Count APCs.

13. Spin down T cells @369*xg* for 4-5 min at 4C. Remove supernatant and resuspend in 10 mL T cell media. Count T cells.

14. Combine APCs and T cells at a 10:1 ratio (you can also do up to a 1:1 ratio if you don't have enough APCs, but we have found the 10:1 APC:T cell ratio yields the best T cell expansion). Add T cell media to bring to 10 mL final volume in T25 flasks. Add 50 IU/mL IL-2. Ideally, plate 1-2e6 T cells and 10-20e6 APCs in an upright T25 flask. For larger T cell preparations, scale up in larger cell culture flasks proportionally.

Day 2: Add media (as needed) and IL-2

2 **1.** If the flasks are starting to change from orange to yellow in color, add a small volume of media to the flask (up to 10mL in a T25). Do not over-dilute the cells - this will slow their growth and can result in cell death. It if okay to let cells grow in orange media.
2. Add 50 IU/mL of IL-2.

Day 4: Add media (as needed) and IL-2

3 **1.** If the flasks are starting to change from orange to yellow in color, add a small volume of media to the flask (up to 10mL in a T25). Do not over-dilute the cells - this will slow their growth and can result in cell death. It if okay to let cells grow in orange media.
2. Add 50 IU/mL of IL-2.

Day 6: Add media (as needed) and IL-2

4 **1.** If the flasks are starting to change from orange to yellow in color, add a small volume of media to the flask (up to 10mL in a T25). Do not over-dilute the cells - this will slow their growth and can result in cell death. It if okay to let cells grow in orange media.
2. Add 50 IU/mL of IL-2.

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