T cell isolation from mouse tissues

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Jun 30, 2021

1 Works for me Share dx.doi.org/10.17504/protocols.io.sqnedve

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ABSTRACT

This protocol outlines the steps for isolating murine T cells from mouse blood, ascites, spleen, and ID8 tumors.

In brief: Spleens were disrupted by mechanical force through a 70mm filter (BD Falcon, ref: 352350) and red blood cells lysed using ACK lysis buffer (Gibco, cat: A1049201). Ovarian tumors were excised and normal adjacent tissue removed. Tumors were minced using a GentleMACS dissociator (Miltenyi), incubated for 10 minutes on a rotator (Miltenyi) at 37°C (medium spin setting), disrupted through a 70mm filter, washed with RPMI containing 5% FBS, and lymphocytes were purified on a 44/67% Percoll gradient (800*xg* at 23°C for 20 minutes; GE Healthcare, cat: 17-0891-01).

DOI

dx.doi.org/10.17504/protocols.io.sqnedve

PROTOCOL CITATION

Kristin Anderson 2021. T cell isolation from mouse tissues. **protocols.io** https://dx.doi.org/10.17504/protocols.io.sqnedve

CREATED

Aug 17, 2018

LAST MODIFIED

Jun 30, 2021

PROTOCOL INTEGER ID

14830

MATERIALS TEXT

MATERIALS

Percoll **Sigma Aldrich Catalog #17-0891-01**

ACK Lysing Buffer **Gibco - Thermo Fisher Catalog #A10492-01**

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

Spleen digestion

1 1. Harvest cells by smashing spleen through a 70um filter.
2. Spin down cells at 369*xg* for 4-5 minutes at 4C.

3. Remove supernatant and lyse red blood cells with 2 mL ACK lysis buffer for 2 minutes at room temperature.
4. Quench lysis using 10mL T cell media.
5. Spin down cells at 369*xg* for 4-5 minutes at 4C.
6. Remove supernatant and resuspend cells in T cell media. Store on ice until use.

Blood and Ascites digestion

2 1. Spin down cells at 369*xg* for 4-5 minutes at 4C.
2. Remove supernatant and lyse red blood cells with 2 mL ACK lysis buffer for 2 minutes at room temperature.
3. Quench lysis using 2mL T cell media. Spin down cells at 369*xg* for 4-5 minutes at 4C.
4. Remove supernatant and lyse red blood cells a second time with 2 mL ACK lysis buffer for 2 minutes at room temperature.
5. Quench lysis using 2mL T cell media. Spin down cells at 369*xg* for 4-5 minutes at 4C.
6. Remove supernatant and resuspend cells in T cell media. Store on ice until use.

ID8 ovary tumor digestion

3 1. Excise tumor from animal and remove normal tissue. Be sure to remove any lymph nodes from tumor! Store in RPMI containing 5% FBS on ice until processing.
2. Transfer tumor tissue into 3mL RPMI+5%FBS in a GentleMACs C tube.
3. Mince tumors using a GentleMACS dissociator (Miltenyi). Use protocol 'm_imptumor_01'.
4. Transfer tissue to a 50 mL conical tube. Add 35 mL of collagenase (pre-warmed to 37C).
5. Put tubes on a MACS Mix Rotator and place in the 37C incubator rotating on medium speed for 10 minutes.
6. Spin down tubes at 369*xg* for 4-5 minutes at 4C.
7. Remove supernatant and resuspend tissue in 5mL RPMI+5%FBS. Smash sample through a 70um filter.
8. Spin down tubes at 369*xg* for 4-5 minutes at 4C.
9. Resuspend pellet in 5mL of 44% Percoll.
10. Underlay with 3-5mL of 67% Percoll.
11. Spin samples for 20 minutes at 809*xg* at room temperature with NO BRAKE.
12. Remove and discard fat layer floating on top. Gently remove the cells at the interface and transfer to a fresh tube.
13. Wash cells with 15mL RPMI+5% FBS. Spin cells at 518*xg* for 10 minutes at 4C.
14. Remove supernatant. Resuspend cells in either RPMI+5% FBS and store on ice until use, or proceed directly to cell staining for FACS.

Citation: Kristin Anderson (06/30/2021). T cell isolation from mouse tissues. https://dx.doi.org/10.17504/protocols.io.sqnedve