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Intravenous injections

Goran Tomic[1]

[1]The Francis Crick Institute

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Goran Tomic

ABSTRACT

Protocol for mouse tail-vein injections of cell lines.

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BEFORE STARTING

I block randomise* the injections when multiple groups/treatments are present (e.g. Ctrl and KO or isotype control/depleting antibody). The goal is for each cage to have all of the cell lines/treatments.

Starting with Cage 1, I inject different cell lines assigned to each mouse within that cage, and then move to the next cage. This way, any bias with cells sitting on ice for extended

periods of time is applied equally across all the cell lines used.

*Block randomisation prevents the situation where all mice within one cage receive the same cell line/treatment. The way I do it: A random number is assigned to each mouse in a cage (block), and the treatments assigned from lowest to highest number within each cage (e.g Ctrl1 to lowest number, Ctrl2 for the second lowest, etc.). Random number is generated using the 'rand' function in Excel, but copied and pasted as 'values' so they would not change every time.

1 Grow the cells 48-72 h before injection (around 70-80% confluent).

2 Wash the cells with PBS and detach with 1 mL trypsin. Stop the reaction with 3-4 mL complete medium (+10% FBS). Aspirate up and down, and filter through a 40 um filter into sterile FACS tubes.

3 Count the cells. Calculate the total required number of cells and aliquot into 1.5 mL eppendorf tubes (or 15 mL tubes if more cells needed)

4 Spin down at RT in a benchtop centrifuge (3 min, 1200 rpm)

5 Remove the supernatant and resuspend the pelleted cells in the appropriate volume of PBS. Transfer to sterile Bijou tubes (for easier handling later)

6 Keep the cells on ice. Place the mice in the heating chamber.

7 Gently pipette the cell suspension up and down with a P1000 before each cage injection. Prepare the injections for all of the mice in the cage, aspirating around 50 uL extra. Just before the injection, mix the cells in the syringe by moving the plunger up and down. Adjust the volume, pick up the mouse, and inject. Repeat the process for each cage.

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