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# Splenocyte Preparation for Immune Reconstitution (Humanisation)

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

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## Abstract

This protocol guides through the steps required to humanise mice with human splenocytes

1 Thaw vial (approx. $50\times10^6$ cells/vial) in water bath 37C for 2-3min (leave crystal)

2 Add cells into 10mL/vial of warm 50% RPMI media + 50% FBS

3 Centrifuge 7min at 300g

4 Combine all vials in 10mL RPMI media + 10% FBS

5 Centrifuge 7min at 300g

6 Resuspend all vials in 10mL RPMI media + 10% FBS + add DNAse-I (typically 40uL/mL if DNAse-I 1mg/mL) at 37C (incubator) for 15-20min (depending on the amount of clumps seen). Check and flick the tube every 5 min

7 Centrifuge 7min at 300g

8 Resuspend in 10mL RPMI media + 10% FBS and filter with 70um filter (white)

9 Count cells:
-If with Neubauer chamber: resuspend 1:2 (10uL cells + 10uL Trypan Blue); Calculation: average of cells per quadrant x 2 (dil) x $10^4$ (cells/mL) x 10 (mL)
-If automatic counter: resuspend 1:10 (10uL cells + 90uL Trypan Blue); Calculation: average of two reads and take live cells x 5 (dil) x 10 (mL)

10 Resuspend pellet in right volume to: $50\times10^6$ cells/mL ($10\times10^6$ cells/200uL=50,000 cells/uL) for NSG mice, or for NSG-dKO mice $100\times10^6$ cells/mL ($20\times10^6$cells/200uL=100,000 cells/uL) with PBS + 2% FBS in universal tubes/epps

11 Freeze leftover cells (max $50\times10^6$cells/vial) with 10% DMSO in FBS (1mL per vial)

12 Take to the animal facility x2 the number of cells and volume needed per donor (in case there are losses due to clumping, dead volumes etc.)

13 Inject 200uL/mouse intraperitoneally

14 Follow humanization weekly by performing tail vein bleeds with heparin (applying red cell lysis buffer x3, 10min on ice) and flow cytometry for the immune markers: hCD45, mCD45, hCD19, hCD3, hCD8, hCD4, 7AAD