



Oct 06, 2022

# Splenocyte isolation from mice

sffschn<sup>1</sup><sup>1</sup>IEO1 *Works for me* Share

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## ABSTRACT

Simple protocol for isolation of spleenocytes from mouse spleen.

## PROTOCOL CITATION

sffschn 2022. Splenocyte isolation from mice. **protocols.io**  
<https://protocols.io/view/splenocyte-isolation-from-mice-chigt4bw>



## KEYWORDS

Mouse, Organ processing, Spleen

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## CREATED

Oct 06, 2022

## LAST MODIFIED

Oct 06, 2022

## PROTOCOL INTEGER ID

70952

- 1 Collect spleens in some PBS
- 2 Place spleen on a 70 µm mesh filter ontop of a 50 mL Falcon

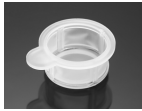
## 70 µm Cell Strainer

Cell Strainer

Falcon 352350



White, Sterile, Individually Packaged



2.1 max. 2 spleens per filter

3 pass through 70 µm mesh filter using a pistol of a 2 mL syringe

4 wash with FACS buffer and top up to 50 mL

 **50 mL FACS buffer**

 **1X PBS (Phosphate-buffered saline )**

 **FCS (Fetal Calf Serum) Life Technologies** 2 %

 **EDTA disodium**

 **dihydrate Abblis Catalog #AB1011793**

1 mM

5 Spin down for 5 min  **00:00:00**

5m

 **400 x g, 4°C, 00:05:00**

## Eppendorf™ 5810R Centrifuge

Centrifuge

Eppendorf 02-262-8187



6 Pour off supernatant


7



5m

Add 1 mL of RBC lysis buffer per spleen and incubate for 5 min at RT

 **1 mL per spleen**

 **00:05:00**

 **Room temperature**

8

Add a bit of FACS buffer and pass through new 70 µm mesh filter into a new 50 mL Falcon

9

Fill up to 50 mL

 **50 mL FACS buffer**

10

 **400 x g, 4°C, 00:05:00**

5m

11

Pour off supernatant

12

Resuspend in 5 mL of FACS buffer per spleen

 **5 mL FACS buffer per spleen**

13

Count cells

Neubauer Improved Haemocytometer  
Counting Chamber  
Counting Chamber

Hawksley AC1000 [↗](#)

Void depth: 0.1 mm

Counting Area: 1mm<sup>2</sup>



[↗](#) 0.4% Trypan blue solution Contributed by users

14  400 x g, 4°C, 00:05:00

5m

15 Resuspend cells to obtain a concentration of 1 x 10<sup>8</sup> cells/mL

[\[M\]](#) 1x10<sup>8</sup> Mass Percent

16 Continue with

[↗](#) EasySep™ Mouse NK Cell Isolation Kit Stemcell

Technologies Catalog ## 19855

isolation of primary mouse NK cells.

for