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Immune Cell Isolation from Mouse Spleen

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ABSTRACT

To isolate immune cells from a mouse spleen before sorting

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
EZ-LINE Cell strainer, 70um Nylon PP, 50 per case	SP104181.SIZE.1CS	Bio Basic Inc.
10% FBS RPMI		

- 1 Prepare 50mL of 10% FBS RPMI media

📄 5 ml FBS

📄 45 ml RPMI



**other media may be used

- 2 Wet both sides of a 70µm filter with media above a 50mL conical (make sure there is some media in the conical)
- 3 Place spleen on filter, use the plunger base of a syringe to mash the spleen on to the filter while pouring media through
- 4 Continue pouring media through filter while mashing until spleen is completely mashed. About 30 to 35mL of media should be used
📄 35 ml
- 5 Keep suspended cells on ice in the 10% FBS RPMI media until preparing for sort
- 6 Spin cells down at 350g for 3 min (pulse to 550g if necessary)
- 7 Remove supernatant

8 Resuspend pellet in 1mL of ACK lysing buffer for 1 min on ice

 00:01:00

9 Add 30mL of media, spin down at 350g for 3 min (pulse to 550g if necessary)



**if pellet is still red, another round of ACK might be needed

 30 ml

10 Stain with desired antibodies, filter with sorting filters, dilute if large pellet



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