

# Isolation of Immune cells from Splenocytes

## Lecture 7

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## Isolation of splenocytes

In order to isolate the splenocytes we should provide the followings

- 1- Complete media (RPMI +Fetal Bovine serum 2%)
- 2- Phosphate Buffer Saline (PBS)
- 3- Red Blood Cells (RBC )lysis
- 4- Centrifuge
- 5- Stomacher to destroy the tissue gently
- 6- Pipette
- 7-Vortex
- 7- Filters (different sizes)
- 8- Tubes (50 ml size)
- 9-Trypan blue stain
- 10- Cell Counter

## Procedure of Isolation of splenocytes

- 1- Put the spleen of the mouse into nylon bags contain 10 ml of complete media
- 2- Smash the spleen by stomacher for 10 minutes
- 3- Turn the content into 50 ml tubes which contain filter to remove the undesirable stuff
- 4- Centrifugate the tubes at 1300 rpm/for 7 minutes at 4C.
- 5- Discard the supernatant
- 5- Add 600 microliter of RBC lysis , vortex it quickly and leave it for 45 seconds only then add 50 ml of media ovel all the content.
- 6- Vortex (shaking) the sample gently then Centrifugate it for 1000 rpm/for 10 minutes at 4C .
- 7- Add 50 ml of PBS
- 8- Check the viability and count the cells by cells counter
- 9- Start then whatever you want of any immune assay.

## Isolation of mononuclear cells from lung

In order to isolate the lung immune cells we should provide the followings

- 1- Complete media (RPMI +Fetal Bovine serum 2%)
- 2- Phosphate Buffer Saline (PBS)
- 3- RBC lysis
- 4-Histopaque
- 5- Centrifuge
- 6- Stomacher (smasher)
- 7-Vortex
- 8- Pipette
- 9- Filters (different sizes)
- 10-Tubes (50 ml size)
- 11-Trypan blue stain
- 12- Cell Counter

Best  
Wishes!

The image features the phrase "Best Wishes!" written in a bold, black, cursive script. The text is set against a white, rectangular background that is tilted at an angle. The words "Best" and "Wishes!" are stacked vertically. The word "Wishes!" is written with a long, sweeping underline that extends across the width of the word. The entire composition is decorated with several small, five-pointed gold stars scattered around the text. The background of the entire image is a light blue gradient.