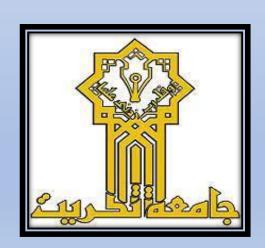
Isolation of Lung Immune components

Lecture 8

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Isolation of monouclear cells from lung Biomedical sciences-Immunology & 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

Procedure for Isolation of Lung Immune cells

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- 1- Put 2 lungs of each couple mouse into bags contain 10 ml of complete media (Because they are few in there number when we isolated them)
- 2- Smash the lung by stomacher for 10 minutes
- 3- Turn the content into 10 ml tubes then filter the content
- 4- Centrifugate the tubes at 1300 rpm/for 7 minutes at 4C.
- 5- Discard the supernatant
- 5- Add 600 microliter of RBC lysis for 45 seconds only and vortex then add 10 ml of media ovel all the content then vortex the tube. Again.
- 6- Centrifugate it for 1000 rpm/for 10 minutes at 4C.

Procedure for Isolation of Lung Immune cells

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- 7-Wash the cells by Adding 5 ml of Phosphate Buffer Saline PBS
- 8- Discard the supernatant then add 5 ml of PBS.
- 9-Layering stage: On another new 10 ml tube, put 5 ml of Hisopaque and leave it for room temprature then pipette the 5 ml of lung cells and added gently and very slowly over the 5 ml histopaque then centrfugate it on 500 xg for 30 minutes and temprature of 25 C
- 10- Collect the middle layer of the tube where the immune cells in this layer then add 10 ml PBS then centrifugate it on 1300 rpm for 7 minutes for washing the cells
- 11- Discard the supernatant
- 12- Add 5 ml of PBS
- 13- Check the viability and count the cells by cells counter
- 14- Start then whatever you want of any immune assay.

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Isolation of mononuclear cells from lung In order to isolate the lung immune cells we should provide the followings

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- 2- Phosphate Buffer Saline (PBS)
- 3- RBC lysis
- 4-Histopaque
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