CELL WORK

Cytotoxicity assay-Tritiated(3H) thymidine

- Effector Cell Preparation

* Any effector cells such as T, NK, macrophage cells or cell lines could be used.
* Here, splenocytes from mice are used.

1. Prepare heparin tubes for blood samples (for FACS) and sterilized tools such as scissors and forceps, dishes, PBS, nylon mash etc (for spleen samples)
2. Obtain blood and spleen samples from the immunized mice
3. Mince spleen in the nylon mash and obtain splenocytes
4. (Optional) to remove red blood cells from splenocytes, use ACK lysis with blood samples
5. Count cells
6. Culture for 5 days in the proper media condition (eg. RPMI, IL-2, Antigen etc)

- Target Cell Preparation

7. Count target cells (eg. B16-F10 melanoma cell line, need 10-12 hr incubation)
8. Remove the culture media
9. Add 3H thymidine media (eg. 50 uCi/ml final) and incubate for the proper time (eg. 8hr)
10. Remove the culture media, and re-suspend cells by the proper cell count with culture media

- 3H thymidine cytotoxic assay

11. Plate effector cells into 96 well plate (round bottom) by serial dilution
12. Add same numbers of 3H thymidine labeled target cells
13. Incubate them (eg 6 hr)
14. Freeze (-80 °C, over 25 min) and thaw (50 °C, 30 min) the plates 3 times
15. Harvest cells using cell harvester and dry up about 5 min (using microwave)
16. Put scintillation bag (general sealing bag could be used, and add cocktail solution (4 ml), and then seal them
17. Counting it using a b-counter