

SOP 05-12 *Herpesvirus saimiri* transformation of macaque T-cells

Purpose and principle:

Method for generating immortalized macaque T-cell lines using a transforming virus.

Materials:

- RPMI1640/10% FBS
- RPMI 1640/10% FBS + 1x b-mercaptoethanol + 20U/ml IL-2
- *Herpesvirus saimiri* stock
- 24-well plates
- PHA-M
- PBS/2%FBS

Procedure:

1. Isolate PBMCs.
2. (Optional) Remove unwanted populations by MACS sort.
3. Resuspend 2×10^6 PBMC in 1.5ml RPMI1640/10% FBS with 5ug PHA-M/ml and transfer to one well of a 24-well plate.
4. Incubate for 2d at 37°C.
5. Remove cells and wash with PBS/2%FBS at 1400rpm for 5m.
6. Resuspend cells in 0.5ml *Herpesvirus saimiri* stock.
7. Incubate for 1h at 37°C.
8. Add 1.5 ml RPMI 1640/10% FBS + 1x b-mercaptoethanol + 20U/ml IL-2 and transfer cells to a clean 24-well plate.
9. Incubate for 4d at 37°C.
10. Remove 1ml media and replace with 1ml RPMI 1640/10% FBS + 1x b-mercaptoethanol + 20U/ml IL-2
11. Continue to split as needed to maintain density $< 1 \times 10^6$ cells/ml

Note: IL-2 requirement for optimal growth may exceed 20U/ml and should be determined for each cell line or clone.

References:

Akari H, Mori K, Terao K, Otani I, Fukasawa M, Mukai R, Yoskikawa Y. *In Vitro* immortalization of Old World Monkey T Lymphocytes with *Herpesvirus Saimiri*: Its Susceptibility to Infection with Simian Immunodeficiency Viruses. *Virology* 1996; 218:382-388.

Goldstein S, Brown CR, Dehghani H, Lisfon JD, Hirsch VM. Intrinsic Susceptibility of Rhesus Macaque Peripheral CD4 T Cells to Simian Immunodeficiency Virus In Vitro Is Predictive of In Vivo Viral Replication. *J. Virology* 2000; 74:9388-9395.

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