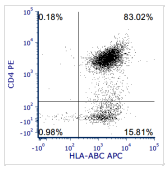

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Activation of Human T cells with Phytohaemagglutinin (PHA)

Zaki Molvi[1]

[1]Immunology Program, Memorial Sloan Kettering Cancer Center, New York, NY

1 Works for me dx.doi.org/10.17504/protocols.io.bpigmkbw

Zaki Molvi

ABSTRACT

Activating T cells with Phytohaemagglutinin (PHA) is a cheap and easy way to initiate a culture of rapidly growing T cells from human samples. PHA-activated T cells, or PHA blasts, can be used for downstream applications such as transduction (e.g. of a CAR or TCR) or as target cells for CTLs since they express sufficiently high levels of HLA class I. An especially useful application is for QCing a batch of frozen PBMCs -- if PHA blasts do not grow in 5 days from a given batch, then it is unlikely that such cells are in adequate condition for more sensitive in vitro applications. I describe here how I like to make them.

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ABSTRACT

Activating T cells with Phytohaemagglutinin (PHA) is a cheap and easy way to initiate a culture of rapidly growing

T cells from human samples. PHA-activated T cells, or PHA blasts, can be used for downstream applications such as transduction (e.g. of a CAR or TCR) or as target cells for CTLs since they express sufficiently high levels of HLA class I. An especially useful application is for QCing a batch of frozen PBMCs -- if PHA blasts do not grow in 5 days from a given batch, then it is unlikely that such cells are in adequate condition for more sensitive in vitro applications. I describe here how I like to make them.

1 Resuspend PBMCs at 2e6 cells/mL in complete medium (e.g. IMDM + 2mM L-Glutamine + 10% FBS). Add PHA to a final concentration of 5-10ug/mL (PHA-L or PHA-P both work in my experience). Incubate for 5 days in an appropriate vessel for your culture size (both flasks and plates will work, even if the latter is not TC coated).

2 Incubate for 5 days at 37C.

3 On day 5-6, growing clusters and acidified media should be visible by naked eye. Count cell density. If density is over 1e6 cells /mL, adjust to 1e6 cell /mL with media. If density is at or below 1e6 cells/mL, spin down half of the culture and resuspend in the same volume with fresh media. Add IL2 to a final concentration of 50U/mL. Incubate for 2-3 days.

4 After 2-3 days, and every 2-3 days thereafter, add IL2 to a final concentration of 50U/mL (always assume all of the IL2 from previous feedings is completely consumed). If cell numbers drop, increase IL2 dose by 50U/mL. Use this increased dose for subsequent feedings. Around day 10-14, restimulate cells with 5-10ug/mL PHA if you will not be freezing them.

5 By day 10, I usually observe that CD4 T cells preferentially grow compared to CD8 T cells (CD4:CD8 of > 70:30), and such CD4 T cells express moderate to high levels of HLA class I, making them ideal for assay of class I-restricted effector cells. Phenotype may change depending on inclusion of other cytokines in addition to IL2, such as IL15 or IL7. Enjoy your PHA blasts!

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