Activation Induced Cell Death Assay

1. Pool ConA blasts and wash in complete media supplemented with a 1:1000 dilution of human recombinant IL-2.
    a. Ficoll histopaque can be used to remove dead cells if desired.
2. After washing, count cells and resuspend at a concentration of $2.5x10^6$ cells/mL in complete media with IL-2.
3. Rest the cells at 37ºC in 5% CO_2 for not more than 3 hours.
4. Plate cells at $5x10^4$ cells/well in triplicate in an anti-CD3 (BD Biosciences, San Diego, CA) coated 96-well microtiter plate starting at 3µg/mL.
    a. Use a 3-fold serial dilution in subsequent wells with the last wells containing PBS as a control.
5. Incubate at 37ºC in 5% CO_2.
6. Add 5mCi tritiated thymidine (ICN Biomedicals Inc- Costa Mesa, CA) to the culture 16 hours before harvest.
7. Harvest cells on a Microplate Scintillation and Luminescence Counter.