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% CO₂ 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% CO₂.
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.