

## Stimulation and Proliferation with PMA/Ionomycin

1. Prepare splenocytes or other tissues according to their Cell Culture protocol.
2. Plate cells in a 96-well plate at a concentration of  $5 \times 10^4$  cells/well with PMA (Sigma) at 10ng/mL and Ionomycin (Sigma) 200ng/mL.
3. Incubate at 37°C in 5% CO<sub>2</sub>.
4. 16 hours prior to harvest, add tritiated thymidine.
5. Use a Microplate Scintillation and Luminescence Counter to harvest the cells.