

BMM Phagocytosis Assay

Day 1 : Jurkat cell culture (grown in RPMI, 10% HIFBS, 1x PS)

- 1- In the afternoon dilute Jurkat cells to 7-8 10^5 cells/ml so that the day after they will be at around 10^6 /ml. These cells will be used for inducing apoptosis.
- 2- Also dilute Jurkat cells to 2-3 10^5 cells/ml so that the day after they will be at around 5 10^5 /ml and thus in their exponential phase. These cells will be used as viable cells.
- 3- Collect BMM by adding PBS/EDTA 10mM for 5min.
- 4- Count the BMM and plate **5 10^4** cells/100 μ l in a 96 wells. Incubate at 37°C 5% CO₂ O/N.

Day 2

- 1- Count Jurkat cells. Put 10 to 20 ml of cells at 10^6 cells/ml in a cell culture dish of 10 cm diameter and irradiate the cells using Stratalinker UV Crosslinker (Stratagene) with the Auto crosslink mode (Energy set at 1200 x 100 μ Joules).
- 2- Incubate the irradiated cells at 37°C 5% CO₂ for 4 hours.
- 3- After 3-4 hours of incubation FACS Jurkat cells using Annexin V and PI to determine the percentage of viable, apoptotic and necrotic cells
- 4- At the same time pellet 10 10^6 apoptotic cells and 10 10^6 viable ones.
- 5- Add 7.5 ml of Calcein 2-5 μ M in PBS to each cell type and incubate 30min at 37°C.
- 6- Pellet the cells, add Jurkat cell medium to a final concentration of 10^6 cells/ml (10 ml) and incubate for 30min at 37°C 5% CO₂
- 7- Pellet the cells and wash twice with 20 ml of Jurkat medium.
- 8- Pellet the cells and adjust the concentration to 5 10^6 cells/ml in BMM medium
- 9- Empty the BMM microplate wells and add 0, 5, 10, 25, 50, and 100 μ l of viable or apoptotic cells. Add medium to a final volume of 100 μ l and give a quick spin to the microplate in order to get Jurkat cells in contact with BMM.
- 10- Incubate for 1 hours at 37°C 5% CO₂
- 11- Read the fluorescence of a determined number of labeled cells in order to establish a standard reference curve.
- 12- Wash the BMM 2-3 times with ice-cold PBS before reading the fluorescence (pipet around the edge of each well as well as in the center).