Preparation of Apoptotic Jurkat Cells for Efferocytosis Assays

We resuspend the desired number of Jurkat cells in 8 mL of PBS and transfer the cell suspension to a petri dish (maximum ~40-50 million cells/dish). Then, we place the dish with cells under a UV lamp for irradiation on a box of tips and tissues so that the distance between the dish and the lamp is ~5 cm. Remember to remove the lid from the petri dish before starting the irradiation. We then irradiate the cells for 15 min in a closed tissue culture hood. Afterwards, we collect the cells (they tend to stick to the plate after irradiation, so pipet up and down well), spin them down, and resuspend them in fresh PBS. Approximately 2-3 hours later, >80% of the cells should be apoptotic (as seen by trypan blue staining) without undergoing necrosis (no blebbing, cells should still be nicely rounded with an intact membrane). At this point, they can be used for the efferocytosis experiment. We generally spin the apoptotic cells down and resuspend them in fresh PBS or medium one more time right before adding to the macrophages, but you could skip this step if you want to add the apoptotic cells together with the molecules that they secrete while undergoing apoptosis.