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pHrodo labeled synaptosome phagocytic assay

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We use this protocol and it's working

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Abstract

pHrodo labeled synaptosome phagocytic assay

1 Appropriate amount of human synaptosomes (SYNs) are thawed and centrifuged at full speed for 3-4 minutes at 4 ºC.

2 Pellet is resuspended and well-mixed in cold 100 mM of sodium bicarbonate, pH 8.5 to a concentration of 1 mg/mL.

3 SYNs are then incubated with amine-reactive pHrodo Red Succinimidyl Ester (SE) dye (Thermo Scientific) for 60 minutes at RT in a twist shaker (Labnet International).

4 Conjugated SYNs are rinsed with DPBS and centrifuged at full speed for 7 times. 10 µg/mL of SYNs were added to hMG plated on a CellCarrier™-96 Ultra Microplate (Perkin Elmer®)

5 Imaged using 20x objective of a Zeiss SP05 confocal microscopy, maintaining constant levels of temperature (37ºC) and CO_2 (5%).

6 Four to five images are taken every minute for 5 hours using the phasecontrast and red fluorescence mode.

7 6 hours LPS pre-stimulated cells are employed as positive phagocytic controls.

8 Phagocytic Index (PI) is quantified using ImageJ, extracting background from the red fluorescent pHrodo.

9 Total fluorescence per image is divided by the total number of cells per image.

10 PI values for every hour of the time lapse are plot and the area under the curve (AUC) is quantified to perform statistical analysis.