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Neuromelanin phagocytosis assay

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Lucas Blasco-Agell^{1,2}, Meritxell Pons-Espinal^{1,2}, Jara Montero-Muñoz^{1,2}, Antonella Consiglio^{1,2}, Miquel Vila³

¹Department of Pathology and Experimental Therapeutics, Bellvitge University Hospital- IDIBELL, 08908 Hospitalet de Llobregat, Spain;

²Institute of Biomedicine of the University of Barcelona (IBUB), Carrer Baldori Reixac 15-21, Barcelona 08028, Spain;

³VHIR-CIBERNED-ASAP

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We use this protocol and it's working

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Abstract

Neuromelanin phagocytosis assay

- 1 One-week microglia (hMG) are plated in plastic 12-wp and placed on a Zeiss AXIOIMAGER Z1 maintaining constant levels of temperature (37 °C) and CO₂ (5%).
- 2 5 ng/mL of NM are added to cultures and 2 images are taken from every well in duplicate every 2 minutes for 16 hours, using the phase-contrast mode.
- 3 Cells are tracked and particles of uptaked NM are counted for every hour.
- 4 Cells that died or moved out of the frame are excluded from the analysis.
- 5 Accumulated NM is plotted during time and AUC is quantified to perform statistical analysis.
- 6 After 24 hours in culture with NM, cell pellets and supernatants of non-stimulated and NM-stimulated cultures are collected and stored at -80 °C for further analyses.