

Materials List for

# Flow Cytometry Approach to Characterize Phagocytic Properties of Acutely-Isolated Adult Microglia and Brain Macrophages In Vitro

Supriya Ramesha<sup>1</sup>, Sruti Rayaprolu<sup>1</sup>, Srikant Rangaraju<sup>1</sup>

<sup>1</sup>Department of Neurology, Emory University

## Corresponding Author

Srikant Rangaraju

srikant.rangaraju@emory.edu

## Citation

Ramesha, S., Rayaprolu, S., Rangaraju, S. Flow Cytometry Approach to Characterize Phagocytic Properties of Acutely-Isolated Adult Microglia and Brain Macrophages In Vitro. *J. Vis. Exp.* (), e61467, doi:10.3791/61467 (2020).

## Date Published

June 23, 2020

## DOI

10.3791/61467

## URL

jove.com/video/61467

## Materials

Name	Company	Catalog Number	Comments
10x Hank's balanced salt solution(10x HBSS)	ThermoFisher	14065056	Store at 4 °C. Used to make SIP as described in the protocol
1x Hank's balanced salt solution(1x HBSS)	ThermoFisher	14175095	Store at 4 °C. Used to make 35% SIP as described in the protocol
1x Phosphate Buffered Saline (PBS)			<a href="https://www.sigmaaldrich.com/technical-documents/protocols/biology/western-blotting/buffers-recipes/10x-phosphate-buffered-saline.html">https://www.sigmaaldrich.com/technical-documents/protocols/biology/western-blotting/buffers-recipes/10x-phosphate-buffered-saline.html</a> Store 10xPBS at Room temperature. Prepare fresh 1xPBS by diluting one part 10xPBS to nine parts of MilliQ dH2O and store it at 4 °C for an hour before use. Make sure the pH of the 1xPBS is 7.0 before refrigeration.
35% SIP			To make 20 mL of 35% SIP, use 7 mL of SIP and 13 mL of ice-cold 1× HBSS. Keep on ice until further processing
APC-Cy7 rat anti-CD11b	BD Pharmingen	557657	Store at 4 °C or keep on ice when in use. Shield from light. Flow cytometry dilution - 1:100
FITC rat anti mouse CD145	BD Pharmingen	553080	Store at 4 °C or keep on ice when in use. Shield from light. Flow cytometry dilution - 1:100
LIVE/DEAD Fixable Blue Dead Cell Stain Kit	ThermoFisher	L34961	Store at -20 °C. Add 50ul of DMSO to one tube of the dye, vortex thoroughly and centrifuge at maximum speed for 1 min. Make 10µL aliquots and store them at -20 °C. Use a fresh aliquot for each experiment and add to the samples at 1:500 dilution.
OneComp eBeads compensation beads	ThermoFisher	01-1111-42	Store at 4 °C, thoroughly vortex the tube before adding to the sample tube(s), keep on ice when in use.

PE-Cy7 rat anti mouse CD145	BD Pharmingen	552848	Store at 4 °C or keep on ice when in use. Shield from light. Flow cytometry dilution - 1:100
Percoll pH8.5-9.5	Sigma	P1644	Store at 4 °C. To make 10 mL of Standard Isotonic Percoll (SIP): Use 9 mL of cold Percoll and 1 mL of cold 10× HBSS.
Phycoerythrin-conjugated microspheres	ThermoFisher	F13083	Store at 4 °C or keep on ice when in use.
β-Amyloid (1 - 42), HiLyte Fluor 488 - labeled, Human	AnaSpec	AS-60479-01	The final concentration of Ab fibrils is 200μM which is then diluted prior to use. Store at -20 °C. Prepare fresh working solution for each experiment.