**Bone Marrow Derived Macrophage (BMDM) differentiation and maintenance.**

Authors: Narayana Yadavalli1,2,3,4,6, and Shawn M. Ferguson1,2,3,4,5,6\*Departments of Cell

1. Biology,Yale University School of Medicine, New Haven, Connecticut 06510, USA.

2. Neuroscience, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

3. Program in Cellular Neuroscience, Neurodegeneration and Repair.

4. Wu Tsai Institute Yale University School of Medicine, New Haven, Connecticut 06510, USA.

5. Kavli Institute for Neuroscience5, Yale University School of Medicine, New Haven, Connecticut 06510, USA.

6. Aligning Science Across Parkinson’s (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA.

**Abstract**

This protocol describes the isolation of bone marrow derived macrophages.

**Keywords**

Macrophages, L929 media, Bone marrow

**Reagents required:**

L929 conditioned media

1% penicillin-streptomycin (Gibco, # 15140-122)

FBS (Gibco, #16140-071)

DMEMF12 (Gibco, #11330-032)

1%GlutaMAXTM (Gibco, # 35050061)

**Procedure**

Note: For mouse bone marrow-derived macrophage (BMDMs) primary cultures, each experiment involved age and sex matched C57BL/6 mice between 3-6 months of age. *Lrrk2* KO (Lrrk2tm1.1Mjff) and G2019S ([*Lrrk2tm1.1Hlme*](http://www.informatics.jax.org/allele/MGI%3A5750894)) homozygous knockin mice were obtained from The Jackson Laboratory76.

1. Mice were euthanized via CO2 or isoflurane inhalation and cervical dislocation.
2. Femurs were collected and cavities were flushed with 5ml ice-cold PBS.
3. Then bone marrow cells were collected by centrifugation 0.3 RCF for 5 minutes.
4. The resulting pellet was resuspended and differentiated for 6 days in culture media containing: DMEMF12 supplemented with 20% FBS, 20% L929 conditioned media, 1% penicillin-streptomycin and 1%GlutaMAXTM.