Required Reagents:

EasySep® Mouse Monocyte Enrichment Kit (19761)

1. Prepare nucleated cell suspension at 1×10^8 cells/mL in recommended buffer (PBS + 2% Fetal Bovine Serum + 1mM EDTA). The maximum start volume per well is 200µl (2x10^7 cells, 96 well plate). The minimum start volume is 50 uL (5x10^6 cells, 96 well plate). Add 5% Rat Serum to cell suspension.

2. Filter cell suspension through a 40uM or 70uM cell strainer if sample is clumpy.

3. Add EasySep® Mouse Monocyte Enrichment Cocktail **50 ul/mL**. Mix well and incubate cells at **4C for 15 minutes**.

4. Wash plate. Top well up to 200µl and spin at 300xg for 7 minutes. Remove supernatant and re-suspend pellet with fresh buffer in original volume.

5. Add EasySep® Mouse anti-Biotin TAC at 60ul/mL. Mix well and incubate cells at **4C for 15 minutes**.

6. Vortex EasySep® D Magnetic particles for 30 seconds to ensure that they are in a uniform suspension.

7. Add particles at **150 µL per mL of cells**. Mix well and incubate at **4C for 10 minutes**.

8. Top up the cell suspension with buffer (refer to table below for required volumes). Mix well, place tube into the magnet and set aside (refer to table below for correct separation times).

Platform	Separation (Top-up vol.)	Separation (# of rounds/min)	Notes
96 well	200µl	1/10’	Untreated U-bottom plates, i.e.CoStar #3788 or BD #351177

9. Carefully pipette the cell suspension into a new Eppendorf tube. ***Do not pour.*** Ensure that the pipettor does not touch the bottom of the wells. The magnetically labelled unwanted cells will remain bound along bottom of the plate, held by the magnetic field of the EasySep® magnet.

10. The enriched cells are now ready for use.