

**Protocol for the use of Monocyte Spin Medium®**



**Monocyte Spin Medium® - Specification**

<b>Product Size</b>	100ml	250ml	500ml
<b>Catalog</b>	60-00095-10	60-00095-11	60-00095-12

**Product Description** Monocyte Spin Medium® is a ready to use, sterile medium for isolation Monocytes out of peripheral blood and buffy coat by a simple centrifugation procedure.

**Other Names** Density medium for the enrichment of Monocytes.

**Directions for use**

1. Check that recommended medium, blood sample, density gradient medium and centrifuge are all at room temperature.
2. Mix Monocyte Spin Medium® thoroughly before use by inverting the bottle several times.
3. Add Monocyte Spin Medium® to tube (see Table).
4. Dilute blood with an equal amount of 1x Wash Buffer (see Table). Catalog 60-00080-10 or other suitable culture medium.

Whole Blood (ml)	Wash Buffer (ml)	Monocyte Spin Medium® (ml)	Tube Size (ml)
1	1	2	15
3	3	3	15
5	5	3	15
15	15	15	50

5. Layer blood on top of Monocyte Spin Medium®, being careful to minimize mixing of blood with Monocyte Spin Medium®.

**Spin**

6. Centrifuge for 15 minutes at 800 x g at room temperature with the **brake off**.

**Collect**

7. Carefully remove the enriched cells from the density gradient medium: plasma interface.
8. After collecting the cells from the interface into a fresh tube – vortex for 5 sec to break up aggregation.

**Wash**

9. Fill up reaction tube with wash buffer.
10. Spin down cells 10 minutes with 300 x g (no or small brake) at 4°C.
11. Pour out supernatant, leave the reaction tube on the table for 20 sec. Wash buffer excess will run down from the tube wall and collect at the bottom.
12. Aspirate most of the liquid above the pellet. The liquid will look foggy, these are mostly platelets – aspiration will improve washing result.
13. Reconstitute pellet with 1 ml of wash buffer by carefully pipetting.
14. Repeat steps 9 to 12.
15. Reconstitute pellet at your desired volume.

