Protocol Leuko Spin Medium

Leuko Spin Medium for the isolation of Leukocytes via densitiy gradient centrifugation.

Directions for use

Check that recommended medium, blood sample, density gradient medium and centrifuge are all at room temperature.

Layer over density gradient medium

1. Dilute sample with Wash Buffer or PBS. See table for recommendations:

Whole Blood (ml)	Wash Buffer (ml)	Density Gradient Medium (ml)	Tube Size (ml)
1	2	2	15
3	6	3	15
5	10	3	15
15	15	15	50

2. Layer the diluted sample on top of the density gradient medium

Spin

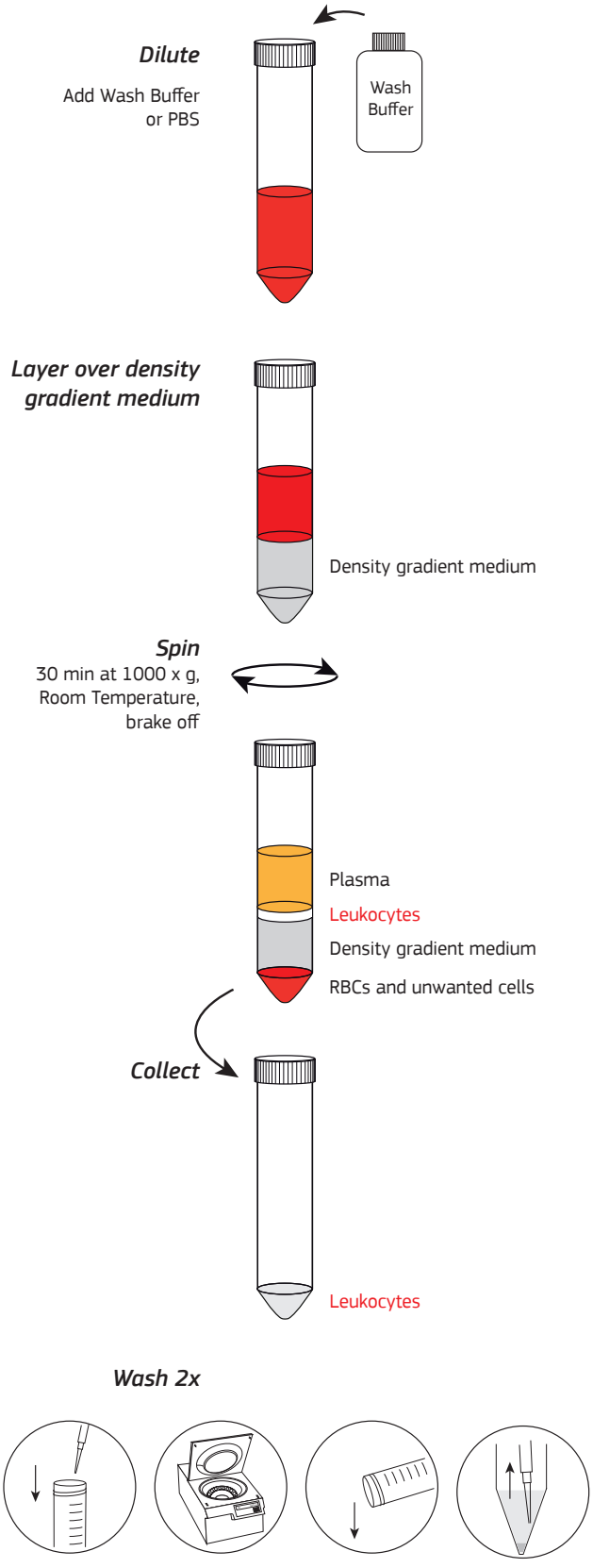
3. Centrifuge for 30 minutes at 1000 x g at room temperature with the **brake off**.

Collect

4. Carefully remove the leukocyte cell fraction from the density gradient medium: plasma interface.
5. After collecting the cells from the interface into a fresh tube – vortex for 5 sec. to break up aggregation

Wash

6. Fill up reaction tube with wash buffer.
7. Spin down cells 10 minutes with 300 x g (no or small brake) at 4°C.
8. Pour out supernatant, leave the reaction tube on the table for 10 sec. Wash buffer excess will run down from the tube wall and collect at the bottom.
9. Aspirate most of the liquid above the pellet. (The liquid will look foggy, these are mostly platelets – aspiration will improve washing result)
10. Reconstitute pellet with 1 ml of wash buffer by carefully pipetting
11. Repeat steps 6 to 9.
12. Reconstitute pellet at your desired volume.



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