pluriSpin Protocol

Required Materials

- Mixing device w/ rotation and tilting, e.g. pluriPlix® (50-01010-80)
- Wash Buffer, e.g. 10x Wash Buffer 500 ml (60-00080-11)
- Density gradient medium*

Directions for use

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

Label

1. Resuspend the pluriSpin® suspension (i.e. vortex 20 sec).
2. Add pluriSpin® suspension at 50 µl/ml of whole blood (e.g. for 4 ml of whole blood, add 200 µl of suspension). Mix well (i.e vortex 2 sec).

Incubate

3. Incubate 15 minutes at room temperature, on pluriPlix® or rolling mixer with tilting and 15 rpm rotation.
4. Dilute sample with an equal volume of wash buffer and mix gently.

Layer over density gradient medium

5. Layer the diluted sample on top of the density gradient medium
   Note: Be careful to minimize mixing of the density gradient medium and sample. See table below for volume recommendations.

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

Spin

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

Collect

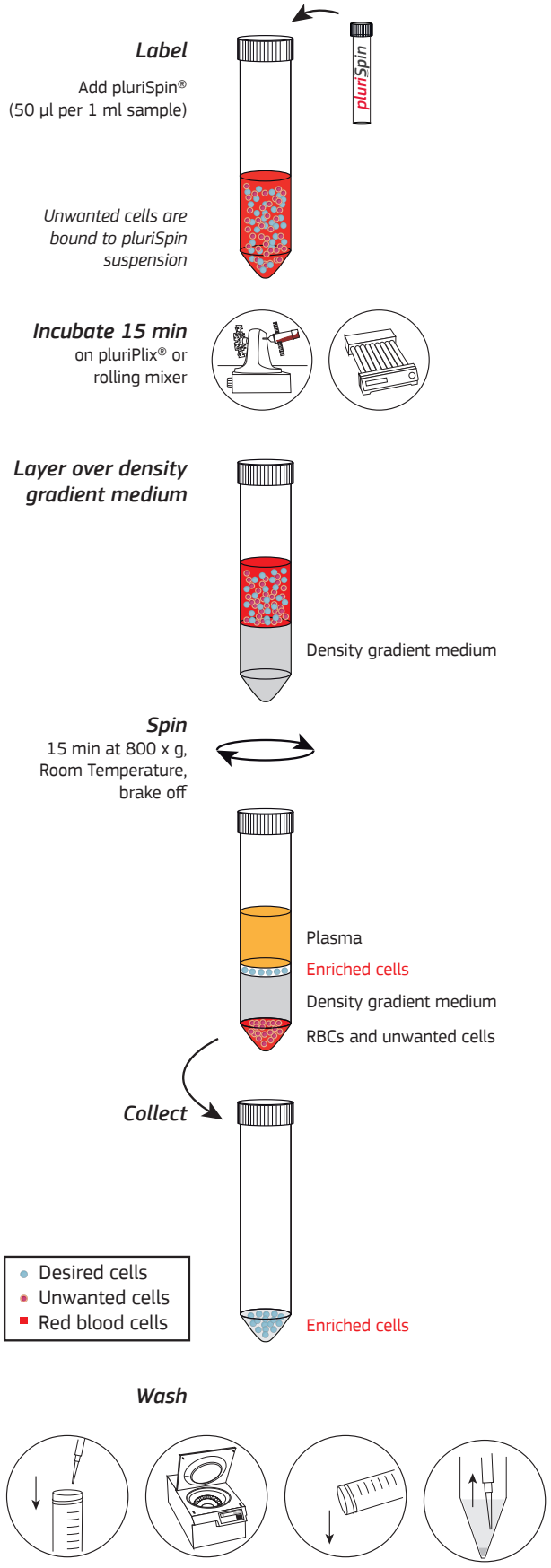
7. Carefully remove the enriched cells from the density gradient medium: plasma interface.
8. After collecting the cells from the interface into a fresh tube – vortex for 5 sec. to break up aggregation

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

Use enriched cells as desired. In case of contamination with red blood cells (RBC) you can lyse these with Ammonium Chloride Solution before flow cytometric analysis. We suggest to use always CD45 staining to identify white blood cells from debris, platelets and RBC.

*Density gradient medium refers to Lymphoprep™, Ficoll-Paque™ PLUS or other similar density gradient media.



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