AQIX® Technology:- PBMC isolation protocol

1. Dilute the Buffy-Coat 1:4 with the AQIX® RS-I/5mM EDTA reagent mix (i.e., 5ml of 0.5M EDTA in 500ml AQIX® RS-I).

2. Distribute 25ml of the diluted Buffy-Coat into 50ml centrifuge tubes.

3. Underlay with 14ml Ficoll-Paque Plus (till red mark of 10ml pipette) by placing the pipette into the tube and removing slowly the pipette boy. Leave the Ficoll running by itself. Gently elevate the pipette and run the Ficoll down till the mark of 2.5ml. Then close the pipette with your finger and take it out completely.

4. Centrifuge 20min at 2250rpm and RTP in a swinging bucket rotor (switch off the brake of the centrifuge!).

5. With a transfer pipette take off the interphase containing lymphocytes (try to take as little Ficoll as possible) and transfer it into another 50ml centrifuge tube. Pool all the interphases from the same buffy-coat in 2 tubes.

6. Centrifuge 5min at 2250rpm and RTP in a swinging bucket rotor (switch of the brake of the centrifuge!).

7. Remove by suction all of the remaining Ficoll above the pellet of cells.

8. Wash off the remaining Ficoll by adding 20ml of the AQIX® RS-I/5mM EDTA reagent mixture and centrifuging for 5min at 2250rpm.

9. Add 15ml of RBC Lysis Buffer to the pellet of cells and incubate at RTP for 5 min.

10. To stop the lysis of the RBC in the pellet of cells add 20ml of the AQIX® RS-I/5mM EDTA reagent mixture and centrifuge for 5min at 2250rpm (with brake on).

11. Pool the 2 pellets in the same tube and wash twice with 45ml AQIX® RS-I (centrifugation at 2250rpm; brake set at 5 for 5 min).

12. Resuspend the pellet in 40ml of medium (AQIX® RS-I+10%FBS+0.1% Pen/Strep+0.1%NEAA+ 5mM b-mercaptoethanol).

13. Count the cells and dilute again the cell suspension if the first cell concentration is too high)

14. Freeze 30 aliquots of $20x10^6$ cells in 1.5ml of freezing medium (70% AQIX® RS-I + 20% FBS + 10% DMSO or, 75% AQIX® RS-I + 20% FBS + 5% glycerol v/w)