

Processing Item Information Sheet (PIIS)

“Viable PBMC Isolation” 2019_R1 Scheme

This sheet contains all the instructions that you should be aware of to conduct the above mentioned Scheme.
Please read carefully before performing any operation.

Processing Instructions

- Schedule and proceed to a blood draw from a healthy donor.
- Use the blood tube of your choice. Any anti-coagulant is permitted. Only 1 blood tube is collected.
- Proceed to the PBMC isolation following your **usual routine PBMC extraction method**.
- Measure the viability (%) of the PBMC isolated and count the viable PBMC isolated (cells/ml).
- Prepare up to 5 aliquots of PBMC. Expected viability and cell concentration are the following: Viability: minimum 80%, Cell concentration: between 5×10^6 and 10^7 cells/ml of medium.
- Proceed to the freezing of the PBMC following your **usual routine method**.

Scheme Specifications

- Please extract **Viable PBMC** following your **usual routine PBMC extraction method**.
- Please be ready to enter the following information:
 - Blood draw information: date and time, anticoagulant, blood volume (ml).
 - PBMC isolation information: Time at start of processing, Type of extraction (Manual, Automatic), Extraction method (density gradient separation (Ficoll, Ficoll-Paque, LymphoPrep), cell preparation tube (CPT), SepMate, Leucoprep, Other), Initial centrifugation speed (g), Initial centrifugation duration (min), Brake (On, Off), Washing medium (HBSS, DPBS, Other), Washing centrifugation speed (g), Washing centrifugation duration (min), Brake (On, Off), Number of washing steps, Suspension medium (RPMI, DPBS, Other), Suspension medium volume (ml), Time at end of processing.
 - PBMC Viability: Method used (Trypan Blue, Flow Cytometry, Impedance, Other), Type of instrument, Viability (%).
 - PBMC Concentration: Type of instrument, Total PBMC (cells/ml), Viable PBMC (cells/ml)
 - Cryopreservation conditions: Cryopreservation medium (Homemade, Commercial), Cryopreservation method (Mr Frosty, Progressive Rate Freezer, Other), Time at start of freezing, Storage temperature before shipment, Number of PBMC aliquots sent to IBBL.
- Please complete the questionnaire as accurately as possible, adding any relevant detail and comment in the appropriate section.

What to Submit

- Once you have extracted **PBMC** from the blood (according to your extraction method), prepare aliquots (1 mL each) of PBMC and freeze them following your routine procedure. Please use your usual tubes to aliquot the PBMC and stick the labels provided with your Laboratory Number, the name of the Scheme (PBMC19R1) and the aliquot number (1 to 5) on each tube.
- All the PBMC aliquots must be shipped **on dry ice** to the following address:
IBBL, PT Programme
Biorepository
1 Rue Louis Rech
L-3555 Dudelange
LUXEMBOURG
(24h emergency call: + 352 621 310 995)
- Please refer to the "Shipment Instructions" for detailed information.
- Your data must be submitted online on the PT website <http://biospecimenpt.ibbl.lu/> using the login information (Laboratory Number and Password) provided to you via email after the registration to the "Viable PBMC Isolation Scheme".
- Please complete the questionnaire as accurately as possible, adding any relevant detail and comment in the appropriate section.

Timelines

<i>Shipment of the extracted PBMC to IBBL</i>	<i>Data Submission</i>	<i>Data analysis & Report preparation</i>	<i>Reports available</i>
<u>Immediately</u> after extraction <u>Before</u> 15 NOV 2019	15 NOV 2019, <u>latest</u>	15 NOV 2019– 17 JAN 2020	28 FEB 2020

In case of doubts in the completion phase, please contact IBBL at ISBERPT@ibbl.lu