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LIFE SCIENCES

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Peripheral Blood Mononuclear Cells (PBMCs) FAQs

How are PBMCs processed?
PBMCs are isolated from whole blood by density gradient centrifugation using SepMate tubes.

Are red blood cells lysed prior to cryopreservation?
Yes, all PBMC samples have residual red blood cells lysed prior to cryopreservation.

What anticoagulant is used?
Inventory PBMCs are collected using sodium heparin as the anticoagulant. Alternative anticoagulants, such as EDTA and sodium citrate, are available upon request.

How are PBMCs cryopreserved?
Inventory PBMCs are cryopreserved in 90% heat-inactivated FBS + 10% DMSO.

What is the recommended storage for cryopreserved PBMCs?
PBMCs are shipped on dry ice. Upon receipt, they should be used immediately or placed in liquid nitrogen vapor phase for long term storage.

How are cell counts and viabilities of PBMCs determined?
Cell counts and viability are determined using a Nexcelom Cellometer® with acridine orange and propidium iodide to identify live and dead nucleated cells, respectively. Unlike dissociated tissue, which is prone to cellular debris, PBMCs can be counted using trypan blue exclusion. If flow cytometry is used to determine cell count and viability, it is recommended that proper FSC thresholds be established to remove platelets, which are present in cryopreserved PBMCs.

How is post thaw QC performed?
Following at least 24 hours in liquid nitrogen, one vial is removed from storage and quickly thawed in a 37°C water bath until only a small frozen crystal remains. Vials are transferred into a biosafety cabinet and diluted with an appropriate volume of DMEM/F12 + 10% FBS in a 15ml conical tube to ensure linearity with the Nexcelom Cellometer. 20µl of the cell suspension is

mixed with 20µl of acridine orange/propidium iodide and counted on the Nexcelom Cellometer to determine cell counts and viability. All cell counts are performed prior to any pelleting and washing of the samples. These counts are estimations of the total live cell yield.

What is the difference between PBMCs and MNCs?
At Discovery Life Sciences, peripheral blood mononuclear cells (PBMCs) notates cells isolated from whole blood, while mononuclear cells (MNCs) represent cells isolated from apheresis material. During the apheresis process, granulocytes, red blood cells, and platelets are reduced in the specimen, and are further removed during the processing. Therefore, MNCs will have drastically reduced levels of platelets compared to whole blood PBMCs.

Do you have any recommendations for culturing PBMCs?
Culturing PBMCs and its derivatives (for example, isolated T cells) needs to be optimized for the specific downstream application. PBMCs are processed aseptically, but sterility is not guaranteed. Therefore, it is highly recommended that antibiotics, such as penicillin and streptomycin, should be used. For more information, please contact info@dls.com.

Are blast percentages available for diseased PBMC and BMMC samples?
Diseased PBMC and BMMC samples are occasionally collected at a different date from the initial sample utilized to establish blast percentages. Therefore, DLS is unable to guarantee blast percentages in diseased samples. However, for AML and multiple myeloma BMMCs, DLS performs flow cytometry following cryopreservation to characterize blast percentages, and these results are available to aid in sample selection.