iba Solutions For Life Sciences

Cell Selection & Expansion

Application Note

FABIAN® - TRACELESS POSITIVE LYMPHOCYTE ISOLATION

Supplementary information

Material and Methods

Blood was obtained from five healthy adult donors and used within 2 h. For manual isolation, PBMCs were prepared using classical density gradient technique. Briefly, 9 ml whole blood were mixed with 9 ml PBS (w/o Ca&Mg; Capricorn), overlayed on a competitor density gradient medium and centrifuged at 400 xg for 30 min at room temperature without brakes. Afterwards, PBMCs were collected from the interface and washed three times with PBS. For automated isolation, $CD81^+$ cells were isolated using Fab-TACS® Auto Columns on the FABian® device (IBA GmbH) from a total amount of 9 ml blood.

Yields and purities of the cell populations were analyzed on a CytoFLEX (BC) flow cytometer using the following antibodies: CD3 (OKT-3), CD4 (OKT-4), CD8 (HIT8a), CD14 (M5E2), CD19 (SJ25C1), CD45 (HI30), CD56 (MEM-188) and CD235a (HI264), either purchased from eBiosciences or Biolegend.

For cytokine determination, a total of $1x10^6$ cells were incubated for 24 h in RPMI 1640 medium, supplemented with 10% heat inactivated fetal bovine serum and 1% Penicillin/Streptomycin (all from gibco™). Cell activation was performed using 12.5 ng/ml PMA and 0.5 µg/ml ionomycin (Sigma-Aldrich). After 24 h of incubation, the supernatant was collected and stored at -80° C until measurement. The cytokine measurement was performed using Cytometric Bead Array (CBA, BD Biosciences). The assay was performed according to the manufacturer's instruction. All samples were analyzed on a BD Accury™ C6 flow cytometer.

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