SHORT PROTOCOL No. 04 I November 2014

Automated Natural Killer Cell Isolation from human peripheral blood mononuclear cells using the Eppendorf ep*Motion*® M5073

Introduction

Positive (selection of desired cells) or negative (depletion of unwanted cells) selection approaches from virtually any cell type can be carried out with Eppendorf ep*Motion* M5073. Positive selection involves labeling of cells of interest via antibodies that specifically bind to a cell surface antigen unique for this cell type and subsequent isolation of a target cell population by magnetic beads that bind the antibody – either via a second antibody or Biotin/Streptavidin interaction. In negative selection, the unwanted cells are targeted for depletion using multiple specific antibodies, which target all cell types except the cell of interest, and again bound to magnetic particles.
Here, we demonstrate a method to isolate Natural Killer (NK) cells in negative selection manner by using the Eppendorf ep*Motion* M5073. ep*Motion* M5073 enables full walk-away automation of magnetic cell separation with minimal hands-on time. Using column-free magnetic separation technology to isolate cells by either positive or negative selection, ep*Motion* M5073 can label and separate up to 12 cell samples at one time.
In this study, column-free EasySep® Human NK Cell Enrichment kit (Stemcell Technologies™) was used for enrichment of NK cells from human peripheral blood mononuclear cells (PBMCs). Non-NK cells, for instance, T cells, B cells, stem cells, dendritic cells, monocytes, and granulocytes, are magnetically labeled by dextran-coated magnetic particles using a cocktail of bispecific Tetrameric Antibody Complexes (TAC) antibodies. Magnetically labeled cells are then separated from unlabeled target cells. The labeled unwanted cells remained in the tube, whereas the negatively selected cells are transferred into new tubes. The isolated, enriched cells are useful in various downstream applications and research such as genetic, epigenetic, gene expression studies and cell functional assays.

Material and Methods

Required equipment

> ep*Motion* M5073
> TS 300 Single channel dispensing tool
> TS 1000 Single channel dispensing tool
> Eppendorf BioSpectrometer® kinetic
> PrepRack
> Rack24
> Reservoir Rack
> Reservoir Rack module TC Eppendorf Safe-Lock
> Reservoir 30 mL

Required consumables

> epT.I.P.S.® Motion 300 µL Filter
> epT.I.P.S. Motion 1000 µL Filter
> Eppendorf Safe-Lock Tubes, 2.0 mL
> Eppendorf MagSep™ Tissue gDNA Kit
> EasySep Human NK Cell Enrichment kit (Stemcell Technologies, order no.: 19055)

Worktable layout

Position	Item
MagSep (TMX)	PrepRack with human PBMC and empty tubes (2nd round separation)
A2	1000 µL Filtertips
B1	Reservoir rack with Human NK Cell Enrichment kit reagents in RR module Eppendorf Safe-Lock Tubes, 1.5 mL (Pos. 1) + Reservoir 30 mL (Pos. 3)
B2	300 µL Filtertips
C1	Rack24 with fresh Eppendorf Safe-Lock Tubes, 2.0 mL

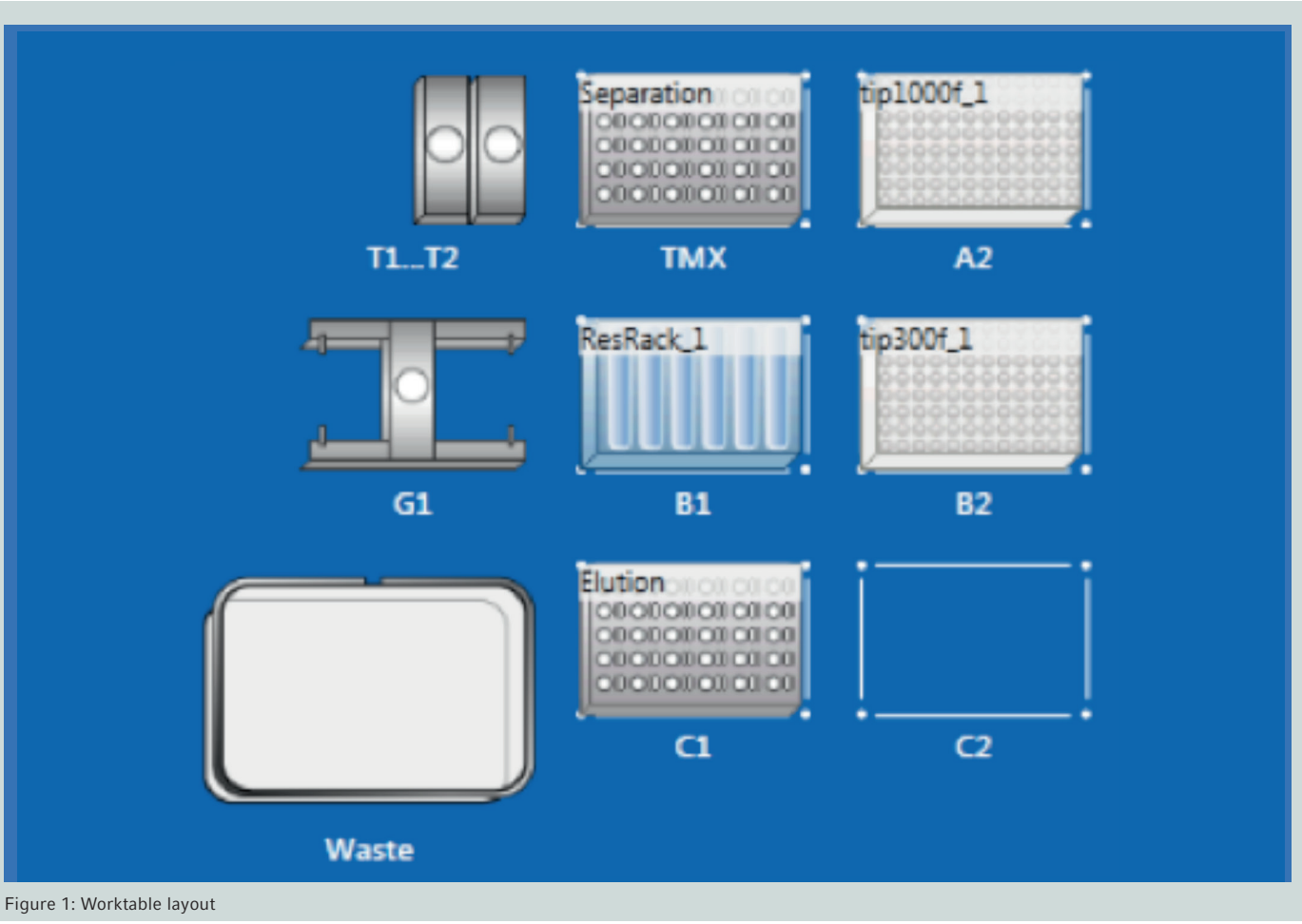

Figure 1: Worktable layout

Reservoir rack layout

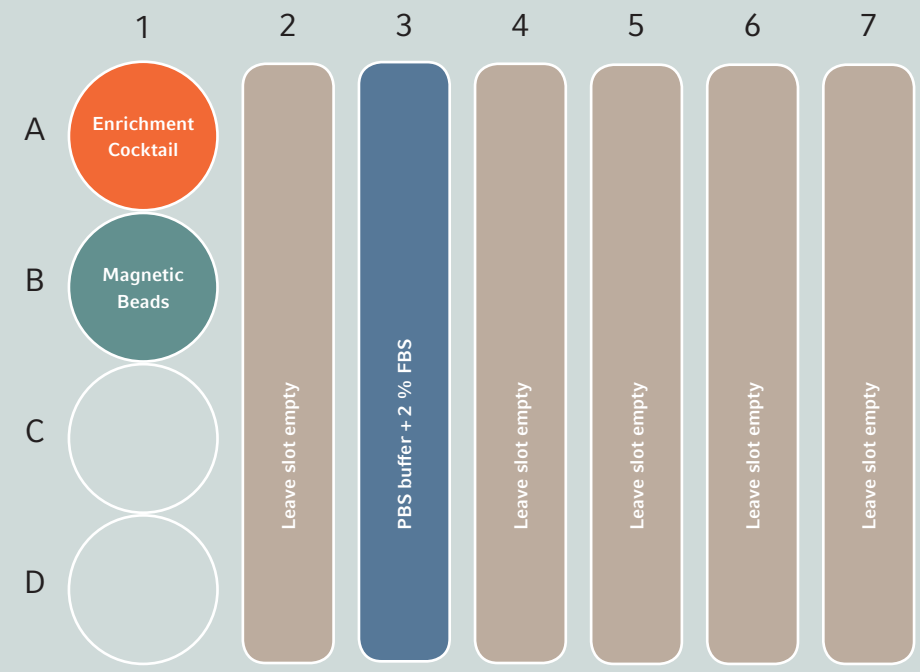

Figure 2: Reservoir rack layout

All the steps in the isolation procedure were carried out in Eppendorf Safe-Lock Tubes, 2.0 mL. Prior to cell isolation, human peripheral blood mononuclear cells (PBMC) were prepared by density gradient centrifugation. The final mononuclear cell fraction was concentrated in 500 µL PBS + 2 % FBS medium and cell number was determined (up to 1.0×10^8 cells). In this study, a cell suspension with a cell count of 2.5×10^7 cells was used. The counted human PBMC cells were placed in Eppendorf Safe-Lock Tubes, 2.0 mL in PrepRack for automated cell isolation. Once the cell samples and the appropriate reagents are loaded onto the instrument, ep*Motion* M5073 automates all the steps necessary to magnetically label and separate the cells.
In this method, 25 µL of the EasySep Human NK Cell Enrichment Cocktail was added to 500 µL cell suspension. The mixture was mixed thoroughly and incubated at room temperature for 15 minutes. 50 µL of the EasySep Magnetic Particles was added, mixed and incubated at room temperature for 10 minutes. The cell suspension was brought up to a total volume of 900 µL by adding PBS + 2 % FBS, the cells were mixed gently prior to transferring to new tubes. The magnetically labeled unwanted cells remained inside the tubes, held by magnetic fingers.

Due to requirement of higher magnetic strength for efficient separation, a 2-step magnetic separation is recommended. The first 12 isolated cell fractions are transferred to 12 fresh tubes for second round of magnetic separation. This step is necessary to increase purity by minimizing risk of carry-over contamination of unwanted cells which bound to magnetic beads.
Up to 12 samples can be isolated in a single run, the samples should be placed and arranged in the PrepRack following the pattern below (a) from position 1 - 12.
The isolated fraction will be transferred to 12 fresh tubes (position 13-24) within same rack, and subject to another round of separation (b).

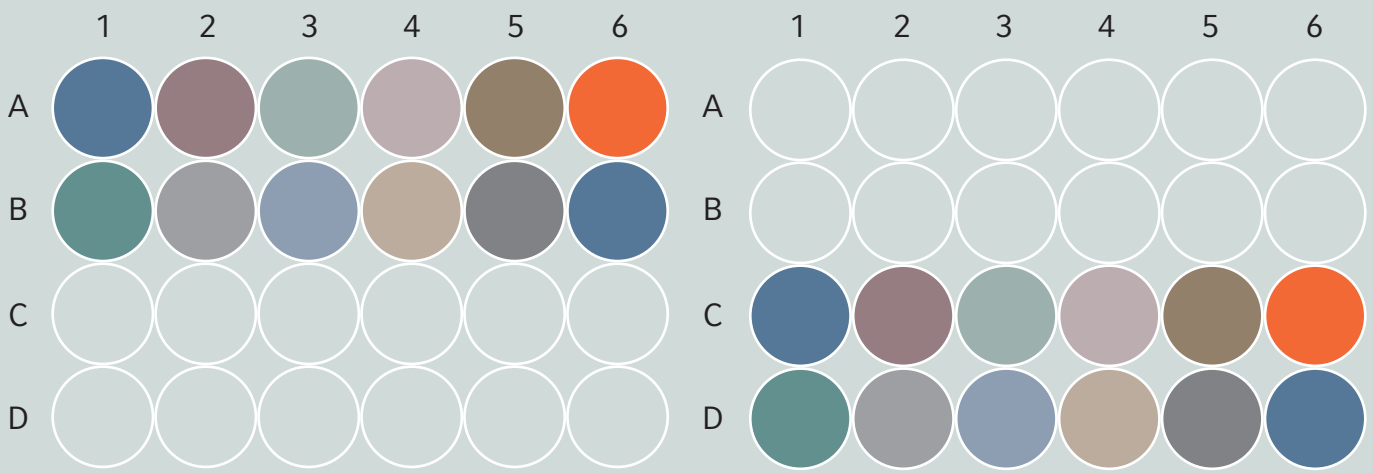

Figure 3: Location of the cell fractions during the two separation steps

Purity Assessment:

After the isolation, cell counting is performed on the negative selected samples to determine the number of cells. 1 x 10^5 cells were used for flow cytometry analysis. The remaining cells were used for gDNA extraction. Purity of NK cells can be measured by flow cytometry after staining with two monoclonal antibody markers, namely Anti-Human CD56 (BD® Biosciences) and Anti-Human CD3 (BD Biosciences) as recommended by kit manufacturer. NK cells are CD56+CD3-. According to the kit manufacturer, the NK cell content of the enriched fraction typically ranges from 73 – 95 %, depending upon the amount of starting materials. Purities may be lower when starting with samples containing less than 10 % NK cells.

DNA extraction of isolated NK cells

DNA from the enriched NK cells is extracted using the Eppendorf MagSep Tissue gDNA kit using same platform. The purified DNA is then quantified using the Eppendorf BioSpectrometer kinetic for determining concentration and purity.

qPCR analysis

The quality of the extracted DNA was further evaluated by performing qPCR for B2M gene. Quantitative real time PCR amplification and melting curve analyses were performed on Eppendorf's Mastercycler® ep realplex System using the KAPA SYBR® FAST qPCR Kit (Kapa Biosystems®).

Results

Results for Cell isolation:

This method is as compatible as the manual conventional method, the purities of NK cells from all samples ranges from 79.1-88.8 %. The yield is considerably good as NK cell is a rare population in PBMC, the normal percentage is around 10 % although it may vary between different individuals and affected by factors such as gender, age, and immunity. This isolation yields NK cells from 1.24-2.34 x10^6 cells which is around 5-10 % of the total PBMC in the beginning.

Running time: 1 hour 10 min

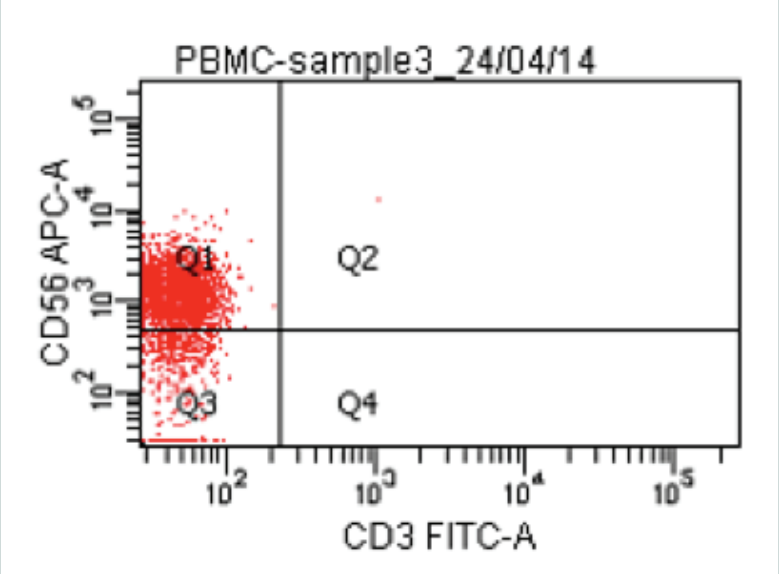

Figure 4: CD56+CD3- (Q1) flowcytometric plots indicated NK cell content from the enriched fractions.

Results for DNA isolation

DNA and purity of isolated NK cells were examined using the Eppendorf BioSpectrometer kinetic, revealing yield recovery ranging from 2.89 – 5.64 µg with A260/280 ratios ranging from 1.79 to 1.86.

Results of qPCR

B2M gene was amplified from 10 ng of purified DNA by qPCR.

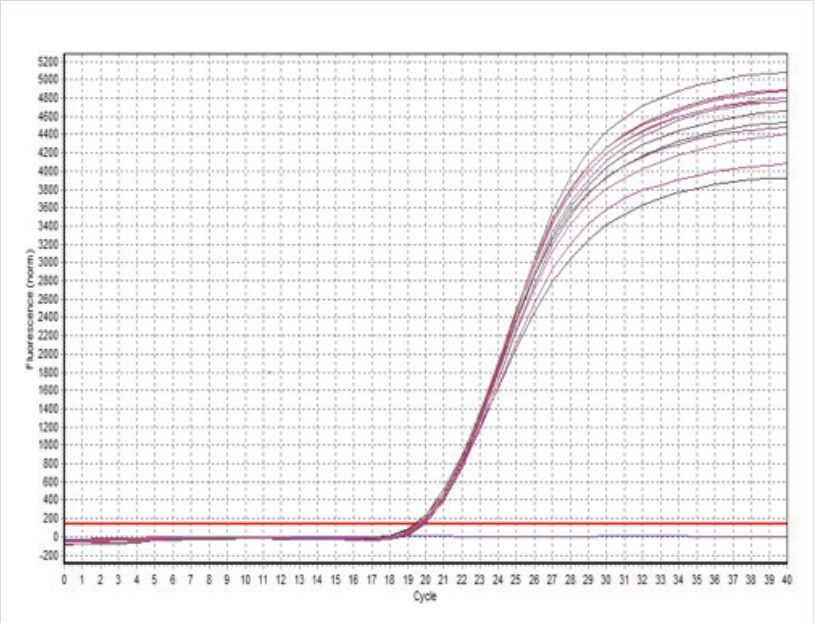

Figure 5: qPCR amplification plots achieved from the purified DNA

Threshold: 139 (Noiseband)
Baseline settings: automatic, Drift correction ON

Ordering Information

Description	Order no. international
Eppendorf ep*Motion*® M5073	5073 000.205
TS 300 dispensing tool	5280 000.037
TS 1000 dispensing tool	5280 000.053
Eppendorf BioSpectrometer® kinetic	6136 000.002
Reservoir rack	5075 754.002
Reservoir rack Module TC Eppendorf Safe-Lock	5075 799.081
epT.I.P.S® Motion 300 µL, PCR clean, sterile	0030 015.231
epT.I.P.S® Motion 1000 µL, PCR clean, sterile	0030 015.258
Reservoir 30 mL	0030 126.505
Eppendorf Safe-Lock Tubes, 1.5 mL	0030 108.051
Eppendorf Safe-Lock Tubes, 2.0 mL	0030 108.078
Eppendorf MagSep™ Tissue gDNA Kit	0030 450.000

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