Staining of survival motor neuron (SMN) protein in peripheral blood mononuclear cells (PBMC) protocol

Noriko Otsuki

Abstract

Functional SMN protein of peripheral blood-derived mononuclear cells was detected with an anti-SMN antibody labeled with a fluorescent dye and analyzed semiquantitatively using intracellular expression intensity and SMN spot formed in cell nucleus as an index, Consider the relationship with the clinical condition and motor function.

Briefly,

1) In order to identify the cell fraction, the fluorescently labeled cell surface antigen-specific antibody is added to the whole blood sample and stained.

2) Hemolyze peripheral blood and fix mononuclear cells.

3) Intracellular staining is performed with SMN protein and nuclear-specific fluorescently labeled antibody.

4) SMN protein expression analysis was performed on a strongly CD33 cell population detected as a cell surface marker, SMN spot detection algorithm was used to analyze the proportion of cells in which intracellular SMN expression and SMN protein aggregated in the nucleus.

Citation: Noriko Otsuki Staining of survival motor neuron (SMN) protein in peripheral blood mononuclear cells (PBMC) protocol. **protocols.io**
https://www.protocols.io/view/staining-of-survival-motor-neuron-smn-protein-in-p-qqkdvuw
Published: 06 Jun 2018

Materials

- Clear Back MTG-001 by Contributed by users
- BD Phosflow™ Lyse/Fix Buffer (5 x) 5558049 by BD Biosciences
- BD Phosflow™ Perm/Wash Buffer I (10 x) 557885 by BD Biosciences
- BD Pharmingen™ Stain Buffer (FBS) 554656 by BD Biosciences
- BD™ CompBeads 51-90-9001229 by BD Biosciences

AF488-anti human SMN mAb by Contributed by users

AF488-MOPC21 by BioLegend

BV610-anti human CD3 mAb by BioLegend

R-PE-anti human CD19 mAb by BioLegend

PE-Cy5-anti human CD33 mAb by Contributed by users

Hoechst 33342 H3570 by Contributed by users

Protocol

Staining cell surface molecules

Step 1.

(1) Dispense 1.5 mL of heparin-treated whole blood into 15 mL_conical tube

(2) Add 30 µL of normal human Ig solution (Clear Back, MTG-001, MBL, Nagoya, Japan)

(3) Gently shake, leave in the dark at room temperature for 15 minutes

(4) Add 5 µL each of PE-HIB19, PE-Cy5-WM53, BV610-UCHT1 (Biolegend)

(5) Gently shake, leave in the dark at room temperature, for 30 minutes

Hemolysis of erythrocytes and immobilization of cell membranes

Step 2.

(6) Warm lysis/fixation buffer and PBS (-) to 37 °C in a water bath

(7) Add 10 mL of pre-warmed lysis/fixation buffer (37 ºC) and mix immediately upside down

(8) Incubate in a water bath at 37 °C for 10 minutes

(9) Mix by inverting, centrifuge (900 × *g*, 5 min)

(10) Discard the supernatant using an aspirator

(11) Resuspended in PBS (-) (37 °C)

(12) Centrifuge (900 × *g*, 5 min)

(13) Discard supernatant

Permeabilaization of transmembrane of cells

Step 3.

(14) Resuspend with 1.5 mL of permeabilization buffer

(15) Incubate at room temperature in the dark for 30 min

(17) Discard supernatant

(18) Resuspended with 1.5 mL of permeabilization buffer

(19) Centrifugation (900 × *g*, 5 min)

(20) Discard supernatant

Cell count and Intracellular staining

Step 4.

(21) Cell count

(22) Dispensing the 1 x 10^6 cells/50 µL in Permeabilization buffer into two microcentrifuge tubes (for stained with specific Ab and isotype control)

(23) Tube for SMN staining: Add 1 µg of AF488-2B1

(24) Tube for isotype control staining: Add 1 test of AF488-MOPC21 (5 µL)

(25) Incubate stained cells (both 23 and 24) at room temperature in the dark for 45 min

(26) Add 500 µL of permeabilization buffer and centrifuge (500 × *g*, 5 min)

(27) Repeat (26) twice, and discard supernatant

Nuclear staining

Step 5.

(28) Suspend in 50 µL of Hoechst 33342 (final concentration: 0.25 µg/mL)

(29) Incubate in the dark at room temperature for 15 min

(30) Add 500 µL of PBS (-) and centrifuged (500 × *g*, 5 min)

(31) Discard supernatant

(32) Suspend with 50 µL of PBS (-)

(33) Analysis