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

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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.

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Protocol status: Working

MATERIALS

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12780

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MATERIALS

☒ Clear Back **Contributed by users Catalog #MTG-001**

☒ BD Phosflow™ Lyse/Fix Buffer (5 x) **BD Biosciences Catalog #5558049**

☒ BD Phosflow™ Perm/Wash Buffer I (10 x) **BD Biosciences Catalog #557885**

☒ BD Pharmingen™ Stain Buffer (FBS) **BD Biosciences Catalog #554656**

☒ BD™ CompBeads **BD Biosciences Catalog #51-90-9001229**

☒ AF488-anti human SMN mAb **Contributed by users**

☒ AF488-MOPC21 **BioLegend**

☒ BV610-anti human CD3 mAb **BioLegend**

☒ R-PE-anti human CD19 mAb **BioLegend**

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

☒ Hoechst 33342 **Contributed by users Catalog #H3570**

Staining cell surface molecules

- 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 memb...

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 - (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 °C) 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

Permeabilization of transmembrane of cells

- 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

- 4
 - (21) Cell count
 - (22) Dispensing the 1×10^6 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 ($500 \times g$, 5 min)
 - (27) Repeat (26) twice, and discard supernatant

Nuclear staining

- 5
 - (28) Suspend in 50 μL of Hoechst 33342 (final concentration: 0.25 $\mu\text{g}/\text{mL}$)
 - (29) Incubate in the dark at room temperature for 15 min
 - (30) Add 500 μL of PBS (-) and centrifuged ($500 \times g$, 5 min)
 - (31) Discard supernatant
 - (32) Suspend with 50 μL of PBS (-)
 - (33) Analysis