Miltenyi Biotec

PSC-mDA Neuron Analysis Kit, anti-human, REAfinity™

Order no. 130-127-439

Contents

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	**60 µL Oct3/4 Antibody, anti-human/mouse, APC, REAfinity** (clone: REA622, isotype: recombinant human IgG1).
	60 µL FoxA2 Antibody, anti-human, APC, REAfinity (clone: REA506, isotype: recombinant human IgG1).
	60 µL OTX2 Antibody, anti-human, Vio® B515, REAfinity (clone: REA1178, isotype: recombinant human IgG1).
	60 µL PAX-6 Antibody, anti-human, PE, REAfinity (clone: REA507, isotype: recombinant human IgG1).
	60 µL TTF-1 Antibody, anti-human, Vio B515, REAfinity (clone: REA1090, isotype: recombinant human IgG1).
	60 µL Sox1 Antibody, anti-human, PE, REAfinity (clone: REA698, isotype: recombinant human IgG1).
	25 mL Fixation/Permeabilization Solution 1
	2×40 mL Fixation/Permeabilization Solution 2
	40 mL Permeabilization Buffer (10×)
Capacity	30 tests and up to 1×10^6 total cells/test.
Product format	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store antibodies and Fixation/Permeabilization Solution 1 and 2 protected from light at 2–8 °C. Do not freeze. Store Permeabilization Buffer (10×) at room temperature. The expiration date is indicated on the vial label.

1.1 Background information

Pluripotent stem cell (PSC) differentiation is a core aspect of PSC research. In the last decade, protocols for the generation of PSC-derived midbrain dopaminergic (mDA) neurons have been established and optimized. Nevertheless, differences in the manufactured cell lots remain and cannot be avoided. Therefore, a quality control step that allows reliable *in vitro* evaluation of the differentiation efficiency is pivotal.

The PSC-mDA Neuron Analysis Kit, anti-human, REAfinity has been developed for *in vitro* phenotyping the identity and purity of mDA neurons. The kit allows the detection of specific regional markers that are expressed already early during differentiation. It can assess the identity of the cells, the cell number of the different sub-populations, and detect non-differentiated cells that might contaminate the culture. Cellular controls specific to other brain regions, such as ventral and dorsal forebrain cells, ventral hindbrain cells, as well as for remnant pluripotent stem cells are necessary for reliable gate definition and detection of contaminating cell populations.

The kit applies recombinant engineered REAfinity Antibodies. REAfinity Antibodies are recombinant antibodies that provide superior lot-to-lot consistency and purity compared to mouse or rat hybridoma-derived, monoclonal antibodies. They have been recombinantly engineered to produce highly specific antibodies that require no FcR blocking step. Additionally, they all have the same IgG1 isotype, requiring less isotype controls.

In summary, this flow cytometric assay results in a quick and reliable qualitative and quantitative analysis during the differentiation process.

1.2 Applications

- Flow cytometry-based quality control for *in vitro* phenotyping of mDA neurons derived from human PSCs.

1.3 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Deionized or distilled water.
- Flow cytometer equipped with a red (640 nm) and a blue (488 nm) laser, e.g., MACSQuant Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) Propidium Iodide Solution (# 130-093-233) for detection of dead cells during flow cytometric analysis.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

▲ For a detailed step-by-step protocol, as well as a comprehensive list of reagent and instrument requirements refer to the application protocol "Flow cytometry-based quality control assay for PSC-derived midbrain dopaminergic neurons" on **www.miltenyibiotec.com/PSC-mDA-neurons**. The application protocol provides information on cell culture, generation of cellular controls, and detailed analysis.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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