Product Numbers: KF37207

IntraCyte^{TM}r-NSC Intracellular FACS Kit

Phenotyping Kit for rat Neural Stem Cells

Kit Description:

The central nervous system (CNS) is comprised of multiple cell types formed through a process of lineage commitment and phenotypic differentiation of neural stem cells (NSC's) into three key cell types; neurons, astrocytes, and oligodendrocytes (1). The expression of differentiation markers associated with neuronal and glial lineages is used to both identify and quantify NSC's.

The addition of epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF or FGF2) causes proliferation of neural stem cells, and retinoic acid (RA) can induce NSC's to differentiate into neurons, oligodendrocytes, or astroglia (2). Many phenotypic markers are used in the study of neural stem cell differentiation (3). Nestin is commonly used to mark nondifferentiated stem/progenitors, beta-tubulin III (Tuj1) identifies neuronal cells, GFAP identifies astroglia, and CNPase, MBP, and the O1/O4 glycolipid antigens identify committed oligodendroglial progenitors and terminally-differentiated oligodendrocytes.

Flow cytometry (FACS) analysis provides a technology that can supply the required phenotypic information, while also providing the necessary accuracy, sensitivity, and robustness. Its multi-parameter capability allows for the rapid detection and enumeration of multiple phenotypes simultaneously on thousands of cells per second (4). This provides a large amount of high content information regarding a population of cells at the single-cell level, and is ideally suited for studies of CNS stem/progenitor cells, which display a strong inherent potential for multi-lineage differentiation.

Neuromics provides FACS phenotyping kits that quantify cultured neural stem cell phenotypes through the fixation, permeabilization, and detection of intracellular antigens by flow cytometry in single cell suspensions. Primary antibodies are included that have been validated for compatibility with FACS analysis as well as for specificity for the target antigen.

The rat neural stem cell kit is intended for the fixation, permeabilization, and immunodetection by indirect staining of intracellular antigens by flow cytometry in single cell suspensions of rat neural stem and progenitors in culture.

Kit Components:

1. Enzymatic cell disassociation solution (20 ml) (store at 21-24°C)

2. Fix solution (125 ml) (store at 4°C)

3. Wash solution (125 ml) (store at 4°C)

4. Block solution (20x) (3 ml) (store at 4°C)

5. Rabbit IgG control (100 ug) (store at 4°C)

6. Ab-1 – Rabbit anti-GFAP affinity purified polyclonal antibody, pre-diluted & ready to use (0.2 ml) (store at 4°C)

7. Ab-2 – Rabbit anti-Nestin affinity purified polyclonal antibody, pre-diluted & ready to use (0.2 ml) (store at 4°C)

8. Ab-3 – Rabbit anti-TUJ1/beta-tubulin III affinity purified polyclonal antibody, pre-diluted & ready to use (0.2 ml) (store at 4°C)

9. Ab-4 – Rabbit anti-Myelin Basic Protein (MBP) affinitypurified polyclonal antibody, pre-diluted & ready to use (0.2 ml) (store at 4°C)

10. Ab-5 – Rabbit anti-CNPAse affinity purified polyclonal antibody, pre-diluted & ready to use (0.2 ml) (store at 4°C)

11. Dilution buffer for secondary antibodies (2 ml) (store at 4°C)

Reagents not Supplied:

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1. Neural stem cells

2. Neural stem cell media and supplements

3. Fluorescent-labeled secondary antibodies

4. Phosphate buffered saline (PBS) pH 7.4

5. Trypan blue solution

Indirect Staining FACS Protocol:

Preparation of single cell suspensions

1. Wash adherent cultured CNS cells with PBS once briefly by aspirating cell culture medium and adding approximately enough PBS to cover the culture surface.

2. Gently dispense approximately enough Enzymatic Cell Dissociation Solution to cover surface (e.g. 5 mls/10 cm dish or 1 ml per 6 well plate), and place in 37°C incubator for 2-10 minutes. Monitor visually using phase-contrast microscopy every 2-3 minutes. The earliest time that cells have begun to lift off the culture surface with a gentle tap of the vessel is usually after 5-10 minutes.

3. Terminate by addition of an equal volume of protein-containing culture medium to the culture, followed by gentle pipetting up and down 3-5 times to dislodge cells.

4. Centrifuge at 200-300g for 10 minutes at 4°C.

5. Resuspend cell pellet in PBS and count using hemacytometer. Viability of trypan blue negative cells should be 90% or more.

Fixation and permeabilization

1. Wash 1 x 10^6 to 5 x 10^6 viable cells in a single-cell suspension using 10-15 mls ice cold PBS by centrifugation at 200 to 300g for 5 minutes. Protein must be removed prior to fixation. BSA or serum for example will interfere with the fixation reaction. A 15 ml conical centrifuge tube is convenient, since subsequent washing volumes of 10-15 mls will be used. Hank's Balanced Salt Solution can also be used.

2. Fix cells by re-suspension in fixative solution and incubate at room temperature for 30 minutes, or overnight at 4°C. Fix 1 x 10^6 to 5 x 10^6 cells per 1.0 ml of

fixative, scaling up volumes for larger cell numbers. Some cells and antigen/antibody combinations require longer fixation times at lower temperatures, while most antigens are efficiently preserved after 30 minutes at room temperature. You should determine the optimal fixation times and temperatures for your cells and antigen/antibody combinations.

3. Wash fixed cells by addition 10-15 mls of PBS and centrifugation at 300-400g for 10 minutes. The formaldehyde and/or organic solvent contained in the fixative must be removed prior to permeabilization. All steps for this point on can be performed at room temperature. After fixation, cells are less dense and therefore require slightly more force to efficiently pellet.

4. Re-suspend in 1-2 mls using Wash Solution. Using a P1000 tip helps in resuspension, and a cell count can be performed as well to determine yield.

5. Most antigens are stable for up to two months when stored at 4°C, while formaldehyde-sensitive epitopes are usually lost almost immediately upon fixation.

FACS staining

1. Wash fixed cells when ready to stain with $\geq$ three volumes of ice cold Hanks by centrifugation at 400 to 500g for 5 minutes. If using low cell numbers increase spin time to 10 minutes to decrease cell losses.

2. Re-suspend at 10^5 to 10^6 cells per test in Wash Solution. It is generally most convenient to stain for FACS using 0.1-0.2 mls per test with 1 x 10^5 to 5 x 10^5cells in each test in a 1.5 ml microfuge tube. 96-well format can also be used, and we recommend using 50 ul with 1 x 10^5 to 5 x 10^5 cells per well.

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3. Block Solution is provided as a 20x stock and should be added after resuspension of cells to a final concentration of 1x. Add Block Solution, mix and incubate for 1 hour at 4 C. The cells can be stored in Block Solution at 4 C for up to 2 months at this point.

4. Carefully label tubes and dispense cells according to a prepared list. Remember to include the appropriate positive and negative controls.

5. Add appropriate concentration of primary Ab or irrelevant IgG control in 1-10 µl then mix gently. Dilute primary Ab as needed prior to addition using Wash Solution. Most primary Abs are effective at 1ug/ml/0.1 x 10^6 cells, however each antibody used should be carefully titered against the particular cells. The IgG control is absolutely required to ensure that the FACS machine is properly colorcompensated and that the primary and secondary Ab fluorescence signals are specific. Without these controls your data may not be reliable.

6. Incubate 60 minutes-overnight at 4°C, then wash cells by addition of 1.0-1.5 mls of Wash Solution and centrifugation at 500g for 5 minutes. Use at least 1 ml per test for 1.5 ml tubes and 0.3 ml per test for 96 well plates. Only a single wash step is usually required, but sometimes washing twice can reduce background. When aspirating, remember to leave a small volume of wash fluid above the cell pellet.

7. Aspirate and resuspend in 0.1 mls/test of Wash Solution that has Block Solution added.

8. Add 10 ul per FACS sample of diluted fluorochrome-conjugated secondary Ab (pre-titering recommended) at in 1-10 ul Wash Solution.

9. Incubate 60 minutes-overnight at 4°C, then wash cells as before with > three volumes of Wash Solution by centrifugation at 500g for 5 minutes.

10. Re-suspend in 0.2 ml per test of PBS. Store in dark at 4°C for up to 1 week prior to FACS analysis.

11. After setting FACS machine with appropriate controls, collect files as large as possible without any gates. Usually 10,000 event files are sufficient, but more may be desirable for phenotypes below 10% abundance in the total cell population.

12. Re-analysis requires setting a gate first to exclude aggregates, necrotic cells and debris.

13. Next analyze the files from your experimental samples by gating on the FSC vs. SSC gated events on each dot plot. Using themIgG control sample, set analytical gates for Nestin, GFAP, TUJ1, MBP, and CNPase to collect phenotype data.

References:

1. Okano H. Stem cell biology of the central nervous system. J Neurosci Res. 2002 Sep 15;69(6):698-707.

2. Robertson MJ, Gip P, Schaffer DV. Neural stem cell engineering: directed differentiation of adult and embryonic stem cells into neurons. Front Biosci. 2008 Jan 1;13:21-50.

3. Steindler DA. Redefining cellular phenotypy based on embryonic, adult, and cancer stem cell biology. Brain Pathol. 2006 Apr;16(2):169-80.

4. Zeigler, F. and Hall, SG. “Isolation of Oligodendroglial Cells From Cultured Neural Stem/Progenitors.” Stem Cell Assays Ed. Mohan C. Vemuri. For the series Methods in Molecular Biology. Ed. John Walker. Humana Press, NJ, 323-333. 2007.

Quality Control:

Rat neural stem cells are fixed, permeabilized, and stained indirectly according to the kit instructions and analyzed on a flow cytometer for FSC and SSC (>50% intact cells) and primary antibody staining above isotype control level.

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