NeuroCult™ Neural Stem Cell Functional Identification Kit

Antibody-Based Neural Cell Identification Kit

Catalog #05716 1 Kit

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Product Description

The NeuroCult™ Neural Stem Cell Functional Identification Kit contains neural lineage-specific antibodies, secondary antibodies and complete protocols for performing indirect immunocytochemistry for the detection of neurons, astrocytes and oligodendrocytes simultaneously (triple labeling) in differentiated mouse, rat, and human central nervous system (CNS) cell samples.

Product Information

PRODUCT NAME	ISOTYPE	CATALOG #	QUANTITY	UNIT SIZE	STORAGE CONDITIONS
Anti-Neuronal Class III β-Tubulin (Clone TUJ1)	Mouse IgG_{2a}	01409	1	0.25 mL	Refer to product label for storage conditions.
Anti-Glial Fibrillary Acid Protein (GFAP)	Rabbit polyclonal	01415	1	0.2 mL	Store in undiluted aliquots. Stable at -20°C for 12 months from date of receipt. Stable at 2 - 8°C for up to 1 month.
Anti-Oligodendrocyte Marker O4 (Clone 81)	Mouse IgM	01416	2	50 µg	Store at 2 - 8°C in undiluted aliquots. Stable for 6 months from date of receipt.
Goat Anti-Mouse IgG (H+L) Antibody, DyLight® 594	Goat polyclonal	10209	1	1 mL	Store at 2 - 8°C in undiluted aliquots. Stable for 12 months from date of receipt.
Goat Anti-Mouse IgM-FITC	Goat polyclonal	10211	1	1 mg	Store powder at 2 - 8°C until opened. Reconstituted antibody is stable at -20°C for 12 months from date of reconstitution.*
Goat Anti-Rabbit IgG (H+L)-AMCA	Goat polyclonal	10214	1	1.5 mg	Store powder at 2 - 8°C until opened. Reconstituted antibody is stable at -20°C for 12 months from date of reconstitution.*

*To extend the shelf life of reconstituted secondary antibodies, add an equal volume of glycerol to make a final concentration of approximately 50% glycerol and store at -20°C.

NOTE: For complete storage instructions for antibodies, please refer to the individual Product Information Sheets.

Directions for Use

1. Centrifuge antibody tubes briefly before opening, to ensure recovery of entire contents.
2. Reconstitute secondary antibodies (Catalog #10211 and 10214) by adding 0.55 mL glycerol (ACS or better grade) and 0.55 mL distilled water.
3. Incubate at room temperature (15 - 25°C) until a clear solution is obtained.
4. Aliquot reconstituted antibodies in small volumes (e.g. 30 µL) and store at -20°C, avoiding exposure to light to prevent photobleaching.

IMMUNOLABELING PROCEDURE TO SIMULTANEOUSLY IDENTIFY NEURONS, ASTROCYTES and OLIGODENDROCYTES

NOTE: Although this protocol is a "triple labeling" procedure, it can also be used when labeling with only 1 or 2 primary antibodies.

A. FIXATION

1. If using cells grown on coverslips, add 1 mL 4% paraformaldehyde (in phosphate-buffered saline [PBS], pH 7.2) to each well of a 24-well plate. Transfer coverslips into 4% paraformaldehyde solution (one coverslip/well, cells facing up) and fix cells at room temperature (15 - 25°C) for 30 minutes.

   OR

   If using cells grown on pre-coated 8-well chamber slides, remove culture medium from each chamber containing differentiated cells (taking care not to remove the entire volume of medium, to avoid exposing unfixed cells to air) and add 0.5 mL 4% paraformaldehyde (in PBS, pH 7.2) directly into the chamber. Incubate at room temperature (15 - 25°C) for 30 minutes.
2. Aspirate paraformaldehyde solution following incubation.
3. Add PBS (pH 7.2) to samples, incubate for 5 minutes and aspirate. Repeat wash procedure 2 times, for a total of 3 washes.

B. PERMEABILIZATION

1. Permeabilize cells by adding 1 mL of 0.3% Triton X-100 (in PBS) to each well or chamber and incubate at room temperature (15 - 25°C) for 5 minutes.
2. Remove Triton X-100/PBS solution by aspiration. Perform 2 x 5-minute washes using PBS.

C. BLOCKING AND LABELING WITH THREE PRIMARY ANTIBODIES

1. Prepare PBS containing 10% goat serum (not included in kit). This will be used as the diluent for the primary antibody.

   NOTE: The type of serum used depends on the host in which the secondary antibody was generated. The secondary antibodies supplied in this kit are generated in goat, therefore the appropriate serum is goat serum.
2. Dilute primary antibody in the appropriate serum-containing diluent according to the table below, to give a working dilution for labeling.

TARGETED ANTIGEN	CATALOG #	DILUTION
Neuronal Class III β-Tubulin	01409	1 in 1000
GFAP	01415	1 in 100
Oligodendrocyte Marker O4	01416	1 in 50

3. Add diluted antibodies to the 24-well plate or chamber slide in a minimum volume of 175 - 250 µL (enough to cover entire coverslip or surface of chamber slide). Place in a hydrating chamber.
4. Incubate at 37°C for 2 hours or at 2 - 8°C overnight.
5. Wash off primary antibody with 3 x 5-minute washes using PBS.

D. SECONDARY STAINING

1. Dilute secondary antibodies in PBS + 2% goat serum (the same serum used as the diluent for the primary antibodies) according to the table below. Add a minimum volume of 250 µL/well to the 24-well plate or chamber slide.

ANTIBODY	CATALOG #	DILUTION	ABSORPTION PEAK (nm)	EMISSION PEAK (nm)
Goat Anti-Rabbit IgG (H+L)-AMCA	10214	1 in 100	350	450
Goat Anti-Mouse IgM-FITC	10211	1 in 100	492	520
Goat Anti-Mouse IgG (H+L) Antibody, DyLight® 594	10209	1 in 200	593	618

2. Incubate secondary antibodies at 37°C for 30 minutes.

   NOTE: Secondary antibodies are sensitive to light and therefore, whenever possible, keep samples in the dark to prevent photobleaching.

3. Wash off secondary antibody with 3 x 5-minute washes using PBS.
4. After the last wash, add distilled water to each well or chamber.

E. MOUNTING

If pre-coated 8-well chamber slides are used:

1. Follow the manufacturer's protocol for removing the chambers from the glass slides. Rinse slides in distilled water in a Coplin jar.
2. Add ~5 µL mounting medium (e.g. FluorSave™ Reagent, EMD Millipore Catalog #345789) in each chamber slot and cover with a 75 mm coverslip, being careful to avoid trapping any air bubbles.

If coverslips are used, on a clean glass coverslip:

1. Add 10 µL mounting medium (e.g. FluorSave™ Reagent, EMD Millipore Catalog #345789). Remove immunolabeled coverslip from 24-well plate and gently tap corner of coverslip to remove excess water.
2. Place coverslip onto mounting medium (cell side down) and be careful to avoid trapping any air bubbles.
3. Visualize immunolabeled cells under a fluorescent microscope using the appropriate filters for each fluorochrome.