



3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sia1.com
sigma-aldrich.com

Product Information

Neural Antigen Sampler Pack

Stock No. CYTO-4

Component Antisera

Monoclonal Antibody To:	Clone	Minimum Titer	Volume	Reference Product No.
Glial Fibrillary Acidic Protein (GFAP)	G-A-5	1:400	0.2ml	G 3893
Neurofilament 200 (phos & non-phos)	N52	1:400	0.2ml	N 0142
Neurofilament 68	NR4	1:400	0.2ml	N 5139
Neurofilament 160	NN18	1:40	0.2ml	N 5264
Parvalbumin	PARV-19	1:2000	0.2ml	P 3088
Synaptophysin	SVP-38	1:200	0.2ml	S 5768

Individual product certificates of analysis are included.
Individual datasheets may be found on our web site, sigma-aldrich.com

Sigma offers sampler packs of neural antigen antisera to give the user the opportunity to screen a number of antibodies before deciding which one is the most suitable for the desired application. Component antisera are identical to the individual catalog products listed. The products are suitable for immunohistochemistry on frozen and formalin-fixed, paraffin-embedded sections)

Storage

For continuous use, store at 2-8 °C up to one month. For extended storage, solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify by centrifugation before use.

Procedure for Indirect Fluorescent Labeling of Frozen Tissue Sections

Materials

1. Isopentane
2. Liquid Nitrogen
3. Embedding medium (non-autofluorescent)
4. Cryostat
5. Clean glass slides and cover slips

6. Acetone in staining jar at ! 20 °C
7. Staining jars
8. Small test tubes
9. Phosphate buffered saline (PBS)
10. Kimwipes, or other absorbent, lint free tissue
11. 90% Glycerol or other non-fluorescing aqueous mounting medium
12. Humid chamber
13. Micropipettes
14. Clear nail polish (if glycerol is used as mounting medium)
15. Fluorescent microscope
16. Primary antibody
17. FITC-labeled second antibody

Method

1. Pre-cool isopentane in liquid nitrogen. Dip freshly dissected tissue blocks (5 mm x 5 mm) quickly into cold isopentane. Aldehyde fixation should be avoided. Frozen tissue blocks may be stored in sealed vials at ! 70 °C in the presence of a few drops of isopentane to prevent drying.
2. Transfer frozen tissue blocks to the chamber of the cryostat and allow temperature to equilibrate for

- approximately 30 minutes.
3. Mount the tissue block on the stub with the embedding medium.
 4. Trim the surface of the block at $-20\text{ }^{\circ}\text{C}$ or temperature found optimal for the particular tissue type.
 5. Cut 3-5 micron sections of frozen tissue. Transfer sections to clean, glass microscope slides and allow to dry at room temperature ($25\text{ }^{\circ}\text{C}$) for 5-16 hours.
 6. Immerse the slides in the pre-cooled acetone ($-20\text{ }^{\circ}\text{C}$) for 20 minutes.
 7. Allow slides to dry briefly at room temperature.
 8. Immerse slides for 10 minutes in staining jar filled with PBS at room temperature.
 9. In small test tube, dilute primary antibody with PBS.
 10. Lay slides flat, section-side up, in humid chamber. Pipette 50-70 μl of diluted antibody to cover the section. Cover chamber and leave slides undisturbed at room temperature or at $37\text{ }^{\circ}\text{C}$ for at least 2 hours. Incubation at $37\text{ }^{\circ}\text{C}$ increases sensitivity without increasing background staining.
 11. Transfer slides to staining jar filled with PBS at room temperature. Replace PBS at least twice at 10 minute intervals.
 12. Dilute FITC-labeled second antibody in small test tube with PBS.
 13. Wipe slides dry around the sections using a Kimwipe. Do not touch sections. Lay slides flat in humid chamber. Pipette 50-70 μl of diluted second antibody to cover the section. Cover chamber and leave slide undisturbed at room temperature or at $37\text{ }^{\circ}\text{C}$ for 1 hour.
 14. Wash slides 3 times in PBS as in step 11.
 15. Place a drop of 90% glycerol or other aqueous mounting medium over the sections and slowly lower coverslip into place, avoiding bubbles. Coverslip may be sealed around edges with clear nail polish.
 16. Examine tissue using a fluorescent microscope.

LPG 8/04

Sigma brand products are sold through Sigma-Aldrich, Inc.
Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications.
Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply.
Please see reverse side of the invoice or packing slip.