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ProductInformation

Cytoskeletal Antisera Sampler Pack
Cytokeratin/Vimentin

Stock No. CYTO-1

Sigma offers sampler packs of cytoskeletal 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.

Component Antisera

Antiserum To:	Host Animal	Volume	Reference Product No.
Cytokeratin 8.13 (Clone K8.13)	Mouse	0.2ml	C 6909
Cytokeratin 8.12 (Clone K8.12)	Mouse	0.2ml	C 7034
Cytokeratin 4.62 (Clone K4.62)	Mouse	0.2ml	C 7159
Keratin	Guinea Pig	0.2ml	K 4252
Vimentin	Goat	0.2ml	V 4630
Vimentin (Clone VIM 13.2)	Mouse	0.2ml	V 5255

Individual product certificates of analysis are included.

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 °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 °C) for 5-16 hours.
6. Immerse the slides in the pre-cooled acetone (–20 °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 °C for at least 2 hours. Incubation at 37 °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 Kim-wipe. 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 °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.

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