



MOUSE ANTI-HUMAN NUCLEOLI MONOCLONAL ANTIBODY

CATALOG NUMBER:	MAB1277
LOT NUMBER:	
QUANTITY:	100 µL
SPECIFICITY:	Gives diffuse nucleolar staining pattern on all human cell types.
IMMUNOGEN:	Human Raji cell nuclei
ISOTYPE:	IgG ₁
APPLICATIONS:	Indirect immunofluorescence: 1:20-1:30. Use 30-100 µL per well/slide. Optimal working dilutions must be determined by the end user.
FORMAT:	Hybridoma supernatant, concentrated by ammonium sulfate. Buffer: PBS with 0.1% sodium azide.
STORAGE/HANDLING:	Maintain at -20°C in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.

Subcellular Particle Suggested Protocol

IMMUNOFLUORESCENCE AND ANTIBODY SCREENING PROCEDURE

Hybridoma supernatants are examined by indirect immunofluorescence on cell preparations of human lymphoid cells. In order to examine many samples in a short period of time, washed cells (wash 2 times in wash buffer at 4°C) at a concentration of 5×10^6 cells/mL in PBS are pipetted dropwise on PTFE- coated printed microscope slides containing ten 5 mm wells/slide. After the cells are allowed to settle to the surface of the glass (10-15 minutes only), the overlying fluid is quickly removed by aspiration and the cells are dried to the slide by a gentle stream of warm air. The slides are then immediately fixed in 2% formaldehyde, ultra-pure, in PBS for 15 minutes at room temperature. After fixation, the slides are rinsed in PBS and placed in acetone at -20°C for 3 minutes to make the cells permeable. After a final rinse in PBS to remove the acetone, the slides are stored in PBS at 4°C indefinitely in covered Coplan jars.

In addition to the lymphoid cultures, normal human epithelial cells can be screened by indirect immunofluorescence microscopy for positive reactions with the hybridoma supernatants. Since the human epithelial cells grow as monolayer cultures, they are plated directly onto the printed microscope slides after trypsinization and allowed to attach and grow overnight at 37°C in complete medium. The following day, the slides are briefly rinsed in PBS to remove the medium and the cells are fixed as described above. In general, the slides are not allowed to air dry either during or after the fixation procedure in order to maintain the cellular integrity and antigenicity of intracellular molecules.

For photographic analysis, viable cell preparations obtained from ficoll-hypaque gradient separations are cytocentrifuged directly onto slides at 1250 rpm for 10 minutes. This procedure flattens the lymphoid cells and greatly improves the visibility of intranuclear and cytoplasmic antigens. Slides prepared in this manner are fixed in the same way directly after cytocentrifugation.

In order to screen the hybridoma supernatants by indirect immunofluorescence, 30-100 µL of each supernatant (optimize for each individual assay) are pipetted on a well of the printed microscope slides using a different tip for each supernatant. After 60 minutes of incubation at 37°C in a humidified chamber, the slides are rinsed 3 times with PBS at room temperature, and again incubated for 30 minutes at 37°C with 20 µL of a 1:20 dilution of fluorescein-conjugated goat anti-mouse IgG antibody (Chemicon AP124F). The slides are then prepared rinsed 3 times with PBS, counterstained with Evans Blue for 5 minutes at room temperature using a freshly prepared solution containing 50 µL of a 1% stock solution of Evans Blue in 80 mL of PBS, rinsed a final time in PBS, and coverslipped using a 1:1 solution of glycerol: PBS. The slides are then examined by epifluorescence microscopy. Since many of the monoclonal antibodies produced a rapidly diminishing fluorescent reaction, exposure times optimally are less than five seconds.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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