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MOUSE ANTI-HUMAN GOLGI ZONE MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB1271

LOT NUMBER:

QUANTITY: 100 µL

SPECIFICITY: Stains golgi zone area in human cells.

ISOTYPE: IgG_1

APPLICATIONS: Indirect immunofluorescence at 1:20-1:30, 30 - 100 µL per slide well.
Optimal working dilutions must be determined by the end user.

FORMAT: Purified culture supernatant.

PRESENTATION: Liquid in PBS. Contains no preservative.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 12 months after date of receipt. Avoid repeated freeze/thaw cycles.

For research use only; not for use as a diagnostic.

28820 Single Oak Drive • Temecula, CA 92590
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Subcellular Particles
Suggested Protocol

IMMUNOFLUORESCENCE AND ANTIBODY AND ANTIBODY SCREENING PROCEDURE

Hybridoma supernatants are examined by indirect immunofluorescence on cell preparations of human lymphnoid 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 x 10 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 slide by a gentle stream of warm air. The slides are then immediately fixed in 2% formaldehyde, ultrapure, 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 lymphnoid 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 incomplete 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 1,250 rpm for 10 minutes. This procedure flattens the lymphnoid 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 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 5 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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