R&D ® Ab

Research & Diagnostic Antibodies

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Anti-human inducible Nitric Oxide Synthase Monoclonal Antibody 2D2-B2

Supplied as the IgG Fraction from Ascites Fluid

MC-5212 Lot # 8719

This IgG Fraction was isolated from ascites fluid contains mouse monoclonal antibody clone 2D2-B2 raised against recombinant hiNOS. The 50 µg of purified IgG fraction has been packaged in 100 µl of 10mg/ml BSA in PBS as carrier protein. This monoclonal antibody has been epitope mapped using 96 overlapping 18 amino acid synthetic peptides which cover the entire 1153 amino acid length of hiNOS and was found to bind to a defined region of the hiNOS sequence, residues 781-798. This monoclonal antibody has been found to stain iNOS in western immunoblots and by immunocytochemistry. This monoclonal antibody was tested for recognition of other NOS isoforms by ELISA, western immunoblotting, and immunocytochemical techniques. It has been found to be a mouse IgG_1 kappa by isotyping.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
hiNOS (781-798)	100
rhiNOS (Type II)	100
heNOS (806-823)	0
rheNOS (Type III)	0
hnNOS (1045-1062)	0
rhnNOS (Type I)	0

Immunofluorescent Staining of Induced Cells

This IgG fraction of monoclonal antibody 2 D2-B2 has been found to stain cells induced to produce iNOS at a concentration of 250ng/ml. The ability of this monoclonal antibody to bind to iNOS in fixed cells was examined using three different cell lines, DLD-1 (a human epithelial cell line), A-172 (a human glioblastoma cell line) and RAW 264.7 (a mouse macrophage cell line). The cells were cultured for 2 days in normal medium and then induced to produce iNOS by treatment for 16 - 20 hours with a cytokine mixture or a cytokine/LPS mixture. Following the treatment, the cells were washed x 4 and fixed in formalin or 100% ice cold acetone. They were reacted for 60 minutes with the mouse monoclonal antibody, and then with FITC-conjugated goat anti-mouse IgG. The immunofluorescent staining pattern was observed using epifluorescent microscopy.

Western Immunoblot

Western immunoblots resulted in a single band being detected at ~ 130 kDa at a concentration of 100 ng/ml.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 7.5% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the mouse monoclonal antibody after preparing a 100 ng/ml solution. Use 2% normal goat serum in TBS/Tween-20 as buffer, and let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 min per wash in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.