R&D ®
Ab

Research & Diagnostic Antibodies

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

Supplied as Ascites Fluid(sterile filtered)

MC-5211 Lot # 8119

This ascites fluid contains mouse monoclonal antibody(2D2-B2) raised against recombinant hiNOS. It 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 specifically hiNOS 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 IgG1 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 monoclonal antibody has been found to stain cells induced to produce iNOS at a 1:1600 dilution. The ability of this monoclonal antibody to bind to iNOS in fixed cells was examined using two different cell lines, 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 hours with cytokine/LPS mixture. Following the treatment, the cells were washed x 4 and fixed in either 70% or 100% acetone. They were reacted for 60 minutes with the ascites fluid, and then with FITC-conjugated goat anti-mouse IgG. The immunofluorescent staining pattern was observed using epifluorescense microscopy.

Western Immunoblot

Western immunoblots resulted in a single band being detected at ~ 130 kDa at a dilution of 1:1000.

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 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the mouse monoclonal antibody after preparing a 1:1000 dilution. Use 2% normal goat serum in 1:5 diluted evaporated goat milk as buffer for the primary antibody. Dilute the condensed goat milk with TBS/Tween-20. 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 1:5 diluted evaporated goat milk (use TBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 3 and then soak the membrane overnight in a fairly large volume of TBS/Tween-20.
7. Develop color using the DAB reaction or the enhanced DAB reaction.

Note: If goat milk is not available(usually it can be found in grocery stores), you can substitue 4% normal goat serum.

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