R&D® Ab

Research & Diagnostic Antibodies

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Anti-human inducible Nitric Oxide Synthase Monoclonal Antibody 5B3-E6

Supplied as the IgG Fraction from Ascites Fluid

MC-5242 Lot # 8102

This IgG Fraction was isolated from ascites fluid contains mouse monoclonal antibody 5B3-E6 raised against recombinant hiNOS. The 50 μgm of purified IgG has been packaged in 100 μl of 10 mg/ml BSA in PBS as carrier protein. This monoclonal antibody has been epitope mapped using 96 overlapping 18 amino acid long synthetic peptides which cover the entire 1153 amino acid length in 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 iNOS in western immunoblots and by immunocytochemistry. Isotyping shows this monoclonal antibody to be a mouse IgG_1 kappa. This monoclonal antibody was tested for recognition of other NOS isoforms by ELISA, western immunoblotting, and immunocytochemical techniques.

Monoclonal Antibody Specificity

Protein	% Cross Reactivity
hiNOS (781-798)	100
rhiNOS (Type II)	100
heNOS (806-823)	0
rheNOS(TypeIII)	0
hnNOS(1045-1062)	0
rhnONS(Type I)	0

Immunofluorescent Staining

This IgG fraction of monoclonal antibody 5B3-E6 has been found to stain cells induced to produce iNOS. 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 40 hours with a cytokine/LPS mixture. Following the treatment, the cells were washed x 4 and fixed in either 70% or 100% acetone. They were reacted for 120 minutes with the IgG fraction, and then with FITC-conjugated goat anti-mouse IgG for 60 minutes. The immunofluorescent staining pattern was observed using epifluorescence microscopy. Specificity for hiNOS has been determined by the lack of immunocytochemical staining of cells known to produce either Type I or Type III NOS.

Western blot

Western blots resulted in a single band being detected at ~ 130 kDa.

Western Blotting Protocol

1. After SDS-PAGE on 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 250 ng/ml solution. Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-mouse IgG antiserum diluted 1:2500 (Note: dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Greater sensitivity may be achieved using ABC techniques.
6. Wash x 5 for 5 min per cycle in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.