R&D ® Ab

Research & Diagnostic Antibodies

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Anti-human inducible Nitric Oxide Synthase Monoclonal Antibody 21C10-1D10

Supplied as Ascites Fluid (Sterile Filtered)

MC-5226 Lot # 7937

This sterile filtered ascites fluid contains mouse monoclonal antibody clone 21C10-1D10 raised against recombinant hiNOS. It has been epitope mapped using 96 overlapping 18 amino acid long 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 25-54. This monoclonal antibody has been found to stain 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 IgG2b kappa by isotyping.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
hiNOS (25-54)	100
rhiNOS (Type II)	100
hnNOS (201-231)	0
rhnNOS (Type I)	0
heNOS (25-54)	0
rheNOS (Type III)	0

Immunofluorescent Staining of Induced Cells

This monoclonal antibody has been found to stain cells induced to produce iNOS at a 1:500 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 40 hours with a cytokine/LPS mixture. Following the treatment, the cells were washed x 4 and fixed in 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 epifluorescent 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 TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the ascites fluid after preparing a 1:1000 dilution. 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-mouse 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 buffer.
7. Develop color using the enhanced DAB reaction.