R&D® Ab

Research & Diagnostic Antibodies

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Anti-human inducible Nitric Oxide Synthase Monoclonal Antibody 2A12-A4

Supplied as Ascites Fluid (Sterile Filtered)

MC-5206 Lot # 8126

This sterile filtered ascites fluid contains mouse monoclonal antibody clone 2A12-A4 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 985-1002. This monoclonal antibody has been found to stain hiNOS and ecNOS 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 (985-1002)	100
rhiNOS (Type II)	100
hnNOS (1256-1273)	0
rhnNOS (Type I)	0
heNOS (1017-1034)	~60
rheNOS (Type III)	~60

Immunofluorescent Staining of Induced Cells

This monoclonal antibody has been found to stain cells induced to produce iNOS at a 1:1000 dilution. 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 16-24 hours with a cytokine mixture or with a cytokine/LPS mixture. Following the treatment, the cells were washed x 3 and fixed in either neutral buffered formalin or 100% ice cold acetone. They were reacted for 60 minutes with the culture supernatant, 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 1:2000.

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 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the ascites fluid after preparing a 1:2000 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-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 buffer.
7. Develop color using the enhanced DAB reaction.