R&D ® Ab

Research & Diagnostic Antibodies

2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 Phone: 702-638-7800

Anti-human inducible Nitric Oxide Synthase Monoclonal Antibody 22E3-2F5

Supplied as Culture Supernatant

MC-5230 Lot # 8114

This culture supernatant contains mouse monoclonal antibody clone 22E3-2F5 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 781-798. 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 IgG1 kappa by isotyping.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
hiNOS (781-798)	100
rhiNOS (Type II)	~85
heNOS (806-832)	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 1:80 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 colorectal epithelial cell line), A-172 (a human glioblastoma cell line), and RAW 264.7 (a mouse macrophage cell line). The cells were cultured, and then induced to produce iNOS. Following the induction, the cells were washed x 4 and fixed in either neutral buffered formalin or 3% freshly prepared formaldehyde. They were reacted for 60 minutes with the culture supernatant, and then with FITC-conjugated goat anti-mouse IgG. The 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 1:40.

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 1:40 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.