

**Anti-human inducible Nitric Oxide Synthase
Monoclonal Antibody 5D5-H7**
Supplied as the IgG Fraction from Ascites Fluid

MC-5247

Lot # 9338

This IgG Fraction was isolated from ascites fluid contains mouse monoclonal antibody 5D5-H7 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 found to stain hiNOS in western immunoblots and by immunocytochemistry. This monoclonal antibody was tested for recognition of other NOS isoforms by ELISA, western blot, and immunocytochemical techniques. It has been found to be a mouse IgG1 kappa by isotyping.

Monoclonal Antibody Specificity

Protein	% Cross Reactivity
rhiNOS (Type II)	100
rheNOS (Type III)	0
rhnNOS (Type I)	0

Immunofluorescent Staining

This IgG fraction of monoclonal antibody 5D5-H7 has been found to stain cells induced to produce iNOS using a dilution of 1.0 µg/ml. 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 carcinoma 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 hours with a cytokine/LPS mixture. Following the treatment, the cells were washed x 4 and fixed with neutral buffered formalin. They were reacted for 60 minutes with the primary antibody, and then with FITC-conjugated goat anti-mouse IgG. The immunofluorescent staining pattern was observed using epifluorescence microscopy. This IgG fraction has also been found to stain freshly fixed tissue and frozen sections by histochemical techniques.

Western blot

Western blots resulted in a single band being detected at ~ 130 kDa at a dilution of 250 ng/ml.

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.