

**Anti-human Insulin-like Growth Factor 1
Monoclonal Antibody**

Supplied as Ascites Fluid (sterile filtered)

MC-2711

Lot # 8156

This ascites fluid contains mouse monoclonal antibody 1F6-3H10 raised against recombinant hIGF-1. This monoclonal has been found to bind specifically to hIGF-1 by indirect ELISA, western immunoblots, and immunocytochemistry. This monoclonal antibody was tested for recognition of insulin, IGF-2, and other growth factors by ELISA techniques. It has been found to be a mouse IgG₁ Kappa by isotyping.

Monoclonal Antibody Specificity

Polypeptide	% Cross Reactivity
hIGF-1	100
hIGF-2	0
Insulin (human)	0
EGF (human)	0
TGF α	0

Indirect ELISA

This monoclonal antibody containing ascites fluid has been found by indirect ELISA to bind to hIGF-1, and titration experiments show the titer to be greater than 1:5000.

Immunofluorescent Staining

This monoclonal antibody has been found to stain cells which produce IGF-1 at a 1:2000 dilution. The cultured cells were washed x 4 and fixed with neutral buffered formalin.

Western Immunoblot

Western immunoblots resulted in a single band being detected at ~ 7.5 kDa at a dilution of 1:5000.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such as 12% 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 mouse ascites after dilution to at least 1:5000 (Note: higher dilutions may be needed). Use 1% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the antibody bind for 1 - 2 hours.
4. Wash x 4 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-mouse IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 1% normal goat serum in TBS/Tween-20. Incubate 1 - 2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 6 for at least 5 min per wash cycle in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.