R&D® Ab
Research & Diagnostic Antibodies
2645 W. Cheyenne Ave, N. Las Vegas, NV 89032 702-638-7800

Anti-human Insulin-like Growth Factor 1 Monoclonal Antibody 6C9-2E5

Supplied as Ascites Fluid (sterile filtered)

MC-2716 Lot # 8163

This ascites fluid contains mouse monoclonal antibody 6C9-2E5 raised against recombinant hIGF-1. This monoclonal has been found to stain specifically hIGF-1 in western immunoblots and to bind to hIGF-1 by indirect ELISA. This monoclonal antibody was tested for recognition of Insulin and other growth factors by ELISA techniques. It has been found to be a mouse IgG_1 Kappa by isotyping

Monoclonal Antibody Specificity

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

Indirect ELISA

This monoclonal antibodies containing ascites fluid has been found by indirect ELISA to bind to hIGF-1. Titration experiments show the titer to greater than 1:4000 by indirect ELISA

Western Immunoblot

Western immunoblots resulted in a single band being detected at ~ 7.5 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 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in 1:5 diluted evaporated goat milk, using TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the ascites after dilution to at least 1:1000 (Note:higher dilutions may be needed). Use 1% normal goat serum in 1:5 diluted evaporated goat milk as buffer for the primary antibody. Dilute the condensed goat milk with TBS/Tween-20. 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 1% normal goat serum in 1:5 diluted evaporated goat milk (useTBS/Tween-20 to dilute the goat milk). Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 3 and then soak the membrane overnight in a fairly large volume of TBS/Tween-20.
7. Develop color using the DAB reaction or the enhanced DAB reaction.