R&D® Ab
Research & Diagnostic Antibodies
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Anti-human Epidermal Growth Factor Monoclonal Antibody Clone 3A3-11H11

Supplied as Culture Supernatant

MC-2575 Lot # 8110

This culture supernatant contains mouse monoclonal antibody clone 3A3-11H11 raised against recombinant hEGF. This monoclonal antibody has been found to stain specifically hEGF by immunocytochemistry and in western blots. This monoclonal antibody was tested for recognition of TGFα 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
EGF(human)	100
TGFα	0
hIGF-1	0
hIGF-2	0

Indirect ELISA

This monoclonal antibody containing culture supernatant has been found by indirect ELISA to bind to hEGF. Titration experiments show the titer to greater than 1:800 by indirect ELISA.

Immunocytochemistry

This monoclonal antibody has been found to stain specifically cell expressing human EGF at a dilution of 1:100. The ability of this monoclonal antibody to bind to hEGF was examined in cells fixed with neutral buffered formalin. The fixed cells were incubated for 20 min with 4% normal goat serum, reacted with 60 min with the diluted monoclonal antibody, and then with FITC-conjugated goat anti-mouse IgG. The immunofluorescent staining pattern was observed using epifluorescence microscopy.

Western Immunoblot

Western immunoblots resulted in a single band being detected at ~ 6.2 kDa at a dilution of 1:100.

Western Blotting Protocol

1. After SDS-PAGE on 15% percent gels and electrophoretic transfer to PVDF membrane, block the membrane overnight with 4% normal goat serum in TBS/Tween-20 buffer as diluent.
2. Wash x 2 with TBS/Tween-20.
3. Apply the culture supernatant after dilution at least 1:100. (Note: higher dilutions may be needed) 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 (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 for 5 min x 4 in TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.