

**Anti-human Epidermal Growth Factor  
Monoclonal Antibody Clone 1B11-3E12**

Supplied as Culture Supernatant

MC-2570

Lot # 8099

This culture supernatant contains mouse monoclonal antibody clone 1B11-3E12 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 TGF $\alpha$  and other growth factors by ELISA techniques. It has been found to be a mouse IgG1 Kappa by isotyping.

**Monoclonal Antibody Specificity**

<b>Polypeptide</b>	<b>% Cross Reactivity</b>
EGF(human)	100
TGF $\alpha$	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 1:400 by indirect ELISA.

**Immunocytochemistry**

This monoclonal antibody has been found to stain specifically cell expressing human EGF at a dilution of 1:50. 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 1:50.

**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 1:50. (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.