EGF Monoclonal Antibody (3A8), Biotin

Product Details	
Size	100 µg
Species Reactivity	Human
Host/Isotope	Mouse / IgG2a
Class	Monoclonal
Type	Antibody
Clone	3A8
Conjugate	Biotin
Immunogen	Recombinant Human EGF
Form	Liquid
Concentration	0.5 mg/mL
Purification	Protein A
Storage buffer	PBS with 4% BSA
Contains	proprietary Preservative
Storage Conditions	-20°C
RRID	AB_2536658

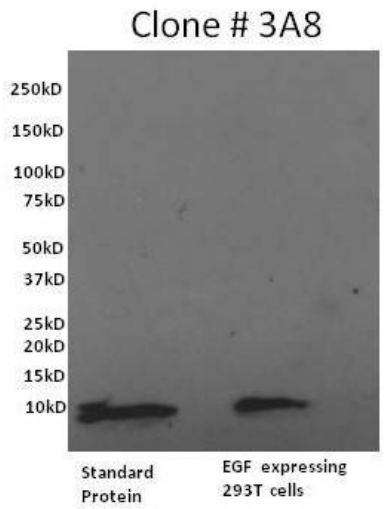
Applications	Tested	Dilution	Published
ELISA (ELISA)	✓	0.125-0.25 µg/mL	
Immunocytochemistry (ICC)	✓	1:50	
Immunofluorescence (IF)	✓	1:50	
Western Blot (WB)	✓	1:1000 - 1:5000	

Product Specific Information

The M806B anti-EGF antibody (biotinylated conjugate of clone 3A8) has successfully been paired as the detection antibody in a sandwich ELISA with coating antibody M805 (Clone 1H11).

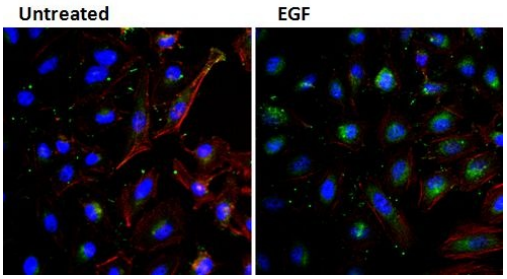
Typical dilutions for sandwich ELISA: Coat = 1-3 µg/mL and Detection = 0.125-0.5 µg/mL.

Product Images For EGF Monoclonal Antibody (3A8), Biotin



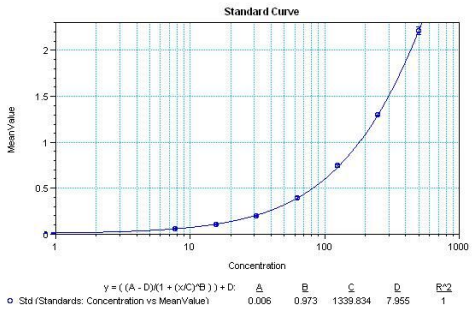
EGF Antibody (M806B) in WB

Western blot analysis of Human EGF was performed by loading 2 µg of recombinant human EGF (Lane 1) or supernatant from an EGF expression clone transfected in 293T cells onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane and blocked with 5% Milk/TBST for at least 1 hour. Membranes were probed with an EGF monoclonal antibody recognizing Human EGF (Product # M806) at a dilution of 1:5000 overnight at 4°C on a rocking platform. Membranes were washed in TBS-0.1%Tween 20 and probed with a goat anti-rabbit-HRP secondary antibody (Product # 31430) at a dilution of 1:10,000 for at least one hour. Membranes were washed and chemiluminescent detection performed using Super Signal West Dura (Product # 34075).



EGF Antibody (M806B) in IF

Immunofluorescent analysis of EGF (green) in HeLa cells either left untreated (left panel) or treated with 10 ng/mL EGF (right panel) for 5 minutes. Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA (Product # 37525) for 15 minutes at room temperature. Cells were probed with an EGF monoclonal antibody (Product # M806) at a dilution of 1:50 for at least 1 hour at room temperature, washed with PBS, and incubated with DyLight 488 goat anti-mouse IgG secondary antibody (Product # 35502) at a dilution of 1:400 for 30 minutes at room temperature. F-Actin (red) was stained with DyLight 554 Phalloidin (Product # 21834) and nuclei (blue) were stained with Hoechst 33342 dye (Product # 62249). Images were taken on a Thermo Scientific ArrayScan at 20X magnification.



EGF Antibody (M806B) in ELISA

Sandwich ELISA analysis of Human EGF was performed using the Thermo Scientific ELISA Kit (Product # EHEGF) by coating a blank 96-well microtiter plate with 100 µL per well of Human EGF moncolonal antibody (Product # M805) in duplicate at 1, 3, 4, 5, 7, and 9 µg/mL in DPBS (Product # 28374) and incubating for 12-18 hours at 4C. The plate was aspirated and blocked with 300 µL per well of 4% BSA and 5% sucrose in DPBS for 1 hour at room temperature. Human EGF recombinant protein at 50 µL per well was added in duplicate at 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 and 0 pg/mL for 2 hours at room temperature along with 50 µL of Human EGF biotinylated monoclonal antibody (Product # M806B) in all applicable wells at 0.2 µg/mL for 2 hours at room temperature. The plate was washed with ELISA Wash Buffer (Product # N503) and incubated with 100 µL per well of Streptavidin-HRP (Product # N504) in all test wells at 1:130,000 dilution for 1 hour at room temperature and then washed and incubated with 100 µL per well of TMB substrate (Product # 34028) for 30 minutes at room temperature in the dark. The plate was stopped with 0.16M sulfuric acid (Product # N600). Absorbances were read on a spectrophotometer at 450-550 nm.