

Anti-AFF4 Picoband™ Antibody (monoclonal, 8G12)

Catalog # ABO15014

Product Information

Application	WB, FC
Primary Accession	Q9UHB7
Host	Mouse
Isotype	Mouse IgG2b
Reactivity	Human
Clonality	Monoclonal
Format	Lyophilized
Description	Anti-AFF4 Picoband™ Antibody (monoclonal, 8G12) . Tested in Flow Cytometry, WB applications. This antibody reacts with Human.
Reconstitution	Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Additional Information

Gene ID	27125
Other Names	AF4/FMR2 family member 4, ALL1-fused gene from chromosome 5q31 protein, Protein AF-5q31, Major CDK9 elongation factor-associated protein, AFF4, AF5Q31, MCEF
Calculated MW	127459
Application Details	Western blot, 0.25-0.5 µg/ml, Human Flow Cytometry, 1-3 µg/1x10 ⁶ cells, Human
Source	Eukaryota
Contents	Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.
Clone Names	Clone: 8G12
Immunogen	A synthetic peptide corresponding to a sequence at the N-terminus of human AFF4, identical to the related mouse sequence.
Purification	Immunogen affinity purified.
Storage	Store at -20 °C for one year from date of receipt. After reconstitution, at 4 °C for one month. It can also be aliquotted and stored frozen at -20 °C for six months. Avoid repeated freeze-thaw cycles.

Protein Information

Name	AFF4
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Synonyms	AF5Q31, MCEF
Function	Key component of the super elongation complex (SEC), a complex required to increase the catalytic rate of RNA polymerase II transcription by suppressing transient pausing by the polymerase at multiple sites along the DNA. In the SEC complex, AFF4 acts as a central scaffold that recruits other factors through direct interactions with ELL proteins (ELL, ELL2 or ELL3) and the P-TEFb complex. In case of infection by HIV-1 virus, the SEC complex is recruited by the viral Tat protein to stimulate viral gene expression.
Cellular Location	Nucleus. Chromosome. Note=Associates to transcriptionally active chromatin but not at snRNA genes {ECO:0000250 UniProtKB:Q9ESC8}
Tissue Location	Ubiquitously expressed. Strongly expressed in heart, placenta, skeletal muscle, pancreas and to a lower extent in brain.

Background

The AFF4 gene encodes a scaffold protein that functions as a core component of the super elongation complex (SEC), which is involved in transcriptional regulation during embryogenesis. The protein encoded by this gene belongs to the AF4 family of transcription factors involved in leukemia. It is a component of the positive transcription elongation factor b (P-TEFb) complex. This gene is mapped to chromosome 5q31.

Images

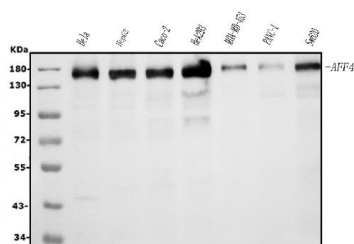


Figure 1. Western blot analysis of AFF4 using anti-AFF4 antibody (M03824).

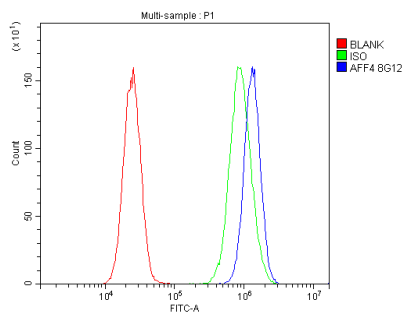
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.

Lane 1: human HELA whole cell lysates,
Lane 2: human HEPG2 whole cell lysates,
Lane 3: human CACO-2 whole cell lysates,
Lane 4: human HEK293 whole cell lysates,
Lane 5: human MDA-MB-453 whole cell lysates,
Lane 6: human PANC-1 whole cell lysates,
Lane 7: human SW620 whole cell lysates.

After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-AFF4 antigen affinity purified monoclonal antibody (Catalog # M03824) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for AFF4 at approximately 150KD. The expected band size for AFF4 is at 150KD.

Figure 2. Flow Cytometry analysis of 293T cells using anti-AFF4 antibody (M03824).

Overlay histogram showing 293T cells stained with M03824 (Blue line).The cells were blocked with 10%



normal goat serum. And then incubated with mouse anti-AFF4 Antibody (M03824, 1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.