

AFP Monoclonal Antibody (P5B8)

Catalog NumberMA5-14666

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human
Host/Isotope	Mouse / IgG1	Published species	Human, Not Applicable
Class	Monoclonal	Tested Applications	
Type	Antibody	ELISA (ELISA)	Assay-dependent
Clone	P5B8	Immunoprecipitation (IP)	Assay-dependent
Immunogen	Purified AFP from Cord Blood	Radioimmune Assays (RIA)	Assay-dependent
Conjugate	Unconjugated	Western Blot (WB)	1:1,000
Form	Liquid	Immunocytochemistry (ICC/IF)	5 µg/mL
Concentration	1 mg/mL	* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.	
Purification	Protein A		
Storage buffer	0.01M potassium phosphate with 0.135M NaCl, 2.5mM KCl, 1mg/mL BSA		
Contains	0.05% sodium azide		
Storage Conditions	Maintain refrigerated at 2-8°C for up to 6 months. For long term storage store at -20°C		

Product specific information

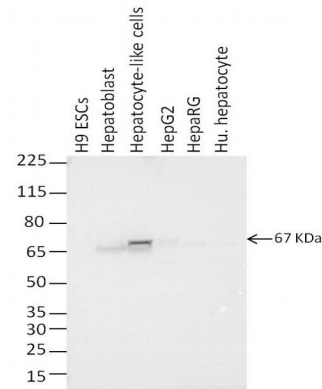
MA5-14666 can be used for immunofluorescence analysis of AFP in the endoderm derived from human embryonic stem cells. By Western blot, MA5-14666 detects endogenous AFP protein in the early hepatocyte-like cells derived from human embryonic stem cells. Product MA514666 is a smaller package size of MIA1305 (formerly sold as a Seradyn product).

Background/Target Information

AFP (Alpha Fetoprotein) is a major plasma protein produced by the yolk sac, intestinal tract and the liver during fetal life. AFP expression in adults is often associated with hepatoma or teratoma. However, hereditary persistence of AFP may also be found in individuals with no obvious pathology. The protein is thought to be the fetal counterpart of serum albumin, and AFP and albumin genes are present in tandem in the same transcriptional orientation on chromosome 4. AFP is found in monomeric, dimeric, and trimeric forms. AFP can also bind to copper ions, nickel ions, fatty acids and bilirubin. The level of AFP in amniotic fluid is used to measure renal loss of protein to screen for spina bifida and anencephaly. High level of serum AFP has been identified in patients with hepatocellular carcinomas (HCC), teratoblastoma, colorectal cancer, pancreatic cancer, and germ cell neoplasms.

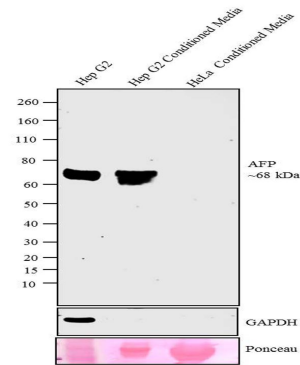
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Product Images For AFP Monoclonal Antibody (P5B8)



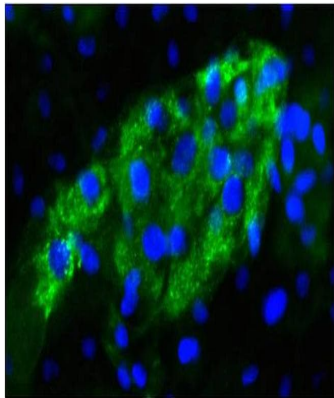
AFP Antibody (MA5-14666) in WB

Western blot analysis of alpha-fetoprotein was performed by loading 10 µg of whole cell extract from H9 ESCs, H9 ESC-derived hepatoblasts, ESC-derived hepatocyte-like cells, HepG2 cells, HepaRG cells, and primary human hepatocytes in reducing conditions and 10 µL Spectra Multicolor Broad Range Protein Ladder (Product # 26634) per well onto a Novex Bolt® 4-12% Bis-Tris Plus Gel with Bolt® MOPS running buffer and Bolt® Antioxidant. Proteins were transferred to a PVDF membrane using the iBlot 2 Dry Blotting System (Product # IB21001), and blocked with 5% non-fat milk in TBST for 1 hour at room temperature. alpha-Fetoprotein was detected at 67 kDa using an alpha-fetoprotein monoclonal antibody (Product # MA5-14666) at a concentration of 1 µg/mL in 5% non-fat milk in TBST overnight at 4°C on a rocking platform, followed by a goat anti-mouse secondary antibody (Product # A24518) at a dilution of 1:10,000 for 1 hour at room temperature. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



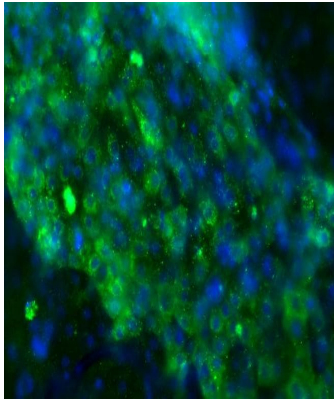
AFP Antibody (MA5-14666)

Antibody specificity was demonstrated by detection of differential basal expression of the target across cell models owing to their inherent genetic constitution. Expression of AFP was observed in HepG2 Conditioned Media compared to HeLa Conditioned Media using AFP Mouse Monoclonal Antibody (Product # MA5-14666) in Western Blot. {RE}



AFP Antibody (MA5-14666) in ICC/IF

Immunofluorescent analysis of alpha-fetoprotein (green) in the endoderm derived from human ES cells. Embryoid bodies (EBs) were generated from the H9 embryonic stem cell line (WiCell Research Institute, WA09) using Gibco® KnockOut™ Serum Replacement. After four days in suspension culture, EBs were plated on Geltrex™-coated tissue culture-treated polystyrene plates and continuously cultured for 21 days. EB cultures were then fixed and permeabilized according to the 3-Germ Layer Immunocytochemistry Kit (Product # A25538) and blocked with 3% BSA for 30 minutes at room temperature. Cells were stained with anti-alpha-fetoprotein (Product # MA5-14666, 1:200 dilution, 5 µg/mL final concentration) at 4°C overnight, and then incubated with a Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:1000 for at least 30 minutes at a room temperature in the dark (green). Nuclei (blue) were stained with Hoechst 33342 (Product # 62249). Stained cells were imaged at 40X using the EVOS® FL Auto Imaging System.



AFP Antibody (MA5-14666) in ICC/IF

Immunofluorescence analysis of alpha-fetoprotein (AFP) (green) in the endoderm derived from human ES cells. Embryoid bodies (EBs) were generated from the H9 embryonic stem cell line (WiCell Research Institute, WA09) using Gibco® KnockOut™ Serum Replacement. After four days in suspension culture, EBs were plated on Geltrex™-coated tissue culture-treated polystyrene plates and continuously cultured for 21 days. EB cultures were then fixed and permeabilized according to the 3-Germ Layer Immunocytochemistry Kit (Product # A25538) and stained with anti-alpha-fetoprotein (Product # MA5-14666, 1:200 dilution, 5 µL/mL final) at 4°C overnight. Secondary staining was completed using Alexa Fluor™ 488-conjugated anti-mouse IgG (Product # A-11001) and DAPI (Product # D1306) for nuclear DNA (blue) for 1 h at room temperature. Stained wells were imaged at 40X using the EVOS® FL Auto Imaging System.

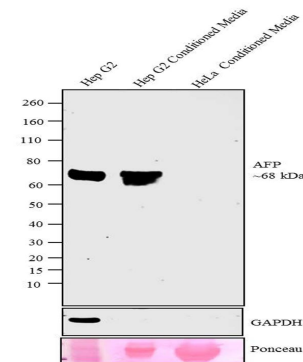
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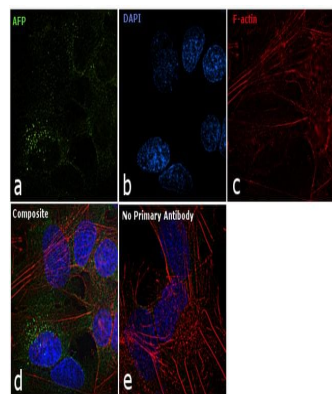
AFP Antibody (MA5-14666) in WB

Western blot analysis was performed on whole cell extracts (30 µg lysate) of Hep G2 (Lane 1), Hep G2 Conditioned Media (Lane 2) and HeLa Conditioned media (Lane 3). The blot was probed with Anti- AFP Mouse Monoclonal Antibody (Product # MA5-14666, 1:1000 dilution) and detected by chemiluminescence using Goat anti Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177, 0.25 ug/ml, 1:4000 dilution). A 68 kDa band corresponding to AFP was detected in Hep G2 conditioned media but not in HeLa conditioned media which is negative for AFP expression.



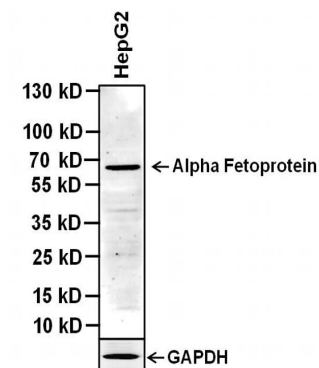
AFP Antibody (MA5-14666) in ICC/IF

Immunofluorescence analysis of AFP was performed using 70% confluent log phase Hep G2 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 1% BSA for 1 hour at room temperature. The cells were labeled with AFP Mouse Monoclonal Antibody (Product # MA5-14666) at 5 µg/mL in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A28175) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing cytoplasmic and Golgi localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.



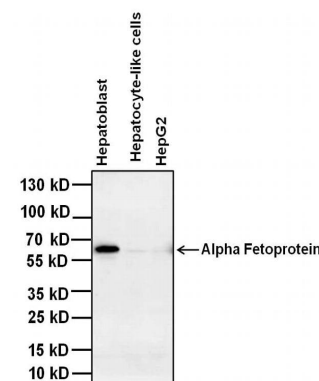
AFP Antibody (MA5-14666) in WB

Western blot analysis of alpha-fetoprotein was performed by loading 20 µg of the indicated whole cell lysates and 5 µL of PageRuler Plus Prestained Protein Ladder (Product # 26619) per well onto a 4-20% Tris-Glycine polyacrylamide gel (Product # WT4202BX10). Proteins were transferred to a nitrocellulose membrane using the G2 Blotter (Product # 62288), and blocked with 5% BSA in TBST for 1 hour at room temperature. Alpha-fetoprotein was detected at ~67 kDa using a alpha-fetoprotein mouse monoclonal antibody (Product # MIA1305) at a concentration of 2 µg/mL in blocking buffer for 1 hour at room temperature on a rocking platform, followed by a Goat anti-Mouse IgG (H+L) Superclonal™ Secondary Antibody, HRP conjugate (Product # A28177) at a dilution of 1:1000 for at least 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34078). GAPDH was detected using a GAPDH rabbit polyclonal antibody, HRP conjugate (Product # PA1-987-HRP), at a dilution of 1:1000 for at least 1 hour at room temperature.



AFP Antibody (MA5-14666) in WB

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