

## DESCRIPTION

<b>Species Reactivity</b>	Human/Mouse
<b>Specificity</b>	Detects human $\alpha$ -Fetoprotein/AFP in direct ELISAs and Western blots.
<b>Source</b>	Monoclonal Mouse IgG <sub>1</sub> Clone # 189502
<b>Purification</b>	Protein A or G purified from hybridoma culture supernatant
<b>Immunogen</b>	Human umbilical cord serum-derived $\alpha$ -Fetoprotein/AFP
<b>Formulation</b>	Lyophilized from a 0.2 $\mu$ m filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 $\mu$ m filtered solution in PBS.

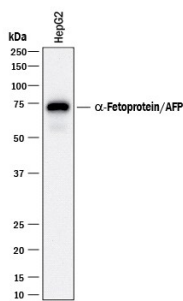
## APPLICATIONS

**Please Note:** Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	<b>Recommended Concentration</b>	<b>Sample</b>
<b>Western Blot</b>	0.5 $\mu$ g/mL	See Below
<b>Immunocytochemistry</b>	8-25 $\mu$ g/mL	See Below
<b>Intracellular Staining by Flow Cytometry</b>	0.25 $\mu$ g/10 <sup>6</sup> cells	See Below
<b>Simple Western</b>	5 $\mu$ g/mL	See Below
<b>CyTOF-ready</b>	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.	
<b>Knockout Validated</b>	$\alpha$ -Fetoprotein/AFP is specifically detected in HepG2 human hepatocellular carcinoma parental cell line but is not detectable in $\alpha$ -Fetoprotein/AFP knockout HepG2 cell line.	

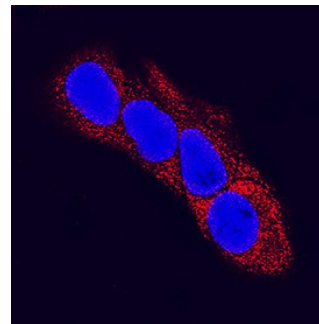
## DATA

### Western Blot



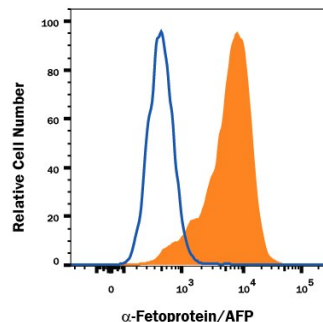
**Detection of  $\alpha$ -Fetoprotein/AFP by Western Blot.** Western blot shows lysates of HepG2 human hepatocellular carcinoma cell line. PVDF membrane was probed with 0.5  $\mu$ g/mL of Mouse Anti-Human/Mouse  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for  $\alpha$ -Fetoprotein/AFP at approximately 70 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

### Immunocytochemistry



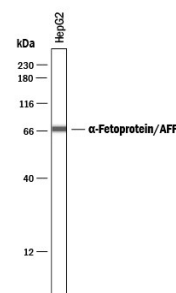
**$\alpha$ -Fetoprotein/AFP in HepG2 Human Cell Line.**  $\alpha$ -Fetoprotein/AFP was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Mouse Anti-Human/Mouse  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368) at 25  $\mu$ g/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Mouse IgG Secondary Antibody (red; Catalog # NL007) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

### Intracellular Staining by Flow Cytometry



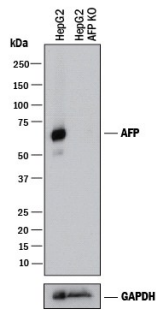
**Detection of  $\alpha$ -Fetoprotein/AFP in HepG2 Human Cell Line by Flow Cytometry.** HepG2 human hepatocellular carcinoma cell line was stained with Mouse Anti-Human/Mouse  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram), followed by Phycoerythrin-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # F0102B). To facilitate intracellular staining, cells were fixed with Flow Cytometry Fixation Buffer (Catalog # FC004) and permeabilized with Flow Cytometry Permeabilization/Wash Buffer I (Catalog # FC005). View our protocol for [Staining Intracellular Molecules](#).

### Simple Western



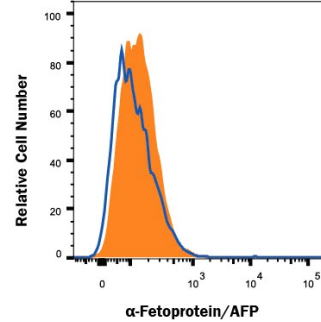
**Detection of Human  $\alpha$ -Fetoprotein/AFP by Simple Western™.** Simple Western lane view shows lysates of HepG2 human hepatocellular carcinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for  $\alpha$ -Fetoprotein/AFP at approximately 70 kDa (as indicated) using 5  $\mu$ g/mL of Mouse Anti-Human/Mouse  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

## Knockout Validated



**Western Blot Shows Human  $\alpha$ -Fetoprotein/AFP Specificity by Using Knockout Cell Line.** Western blot shows lysates of HepG2 human hepatocellular carcinoma parental cell line and  $\alpha$ -Fetoprotein/AFP knockout HepG2 cell line (KO). PVDF membrane was probed with 0.1  $\mu$ g/mL of Mouse Anti-Human/Mouse  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF018). A specific band was detected for  $\alpha$ -Fetoprotein/AFP at approximately 70 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

## Knockout Validated



**$\alpha$ -Fetoprotein/AFP Specificity is Shown by Flow Cytometry in Knockout Cell Line.**  $\alpha$ -Fetoprotein/AFP knockout HepG2 hepatocellular carcinoma cell line was stained with Mouse Anti-Human  $\alpha$ -Fetoprotein/AFP Monoclonal Antibody (Catalog # MAB1368, filled histogram) or isotype control antibody (Catalog # MAB002, open histogram) followed by PE-conjugated Goat anti-Mouse IgG Secondary Antibody (Catalog # F0102B). No staining in the  $\alpha$ -Fetoprotein/AFP knockout HepG2 cell line was observed. View our protocol for [Staining Membrane-associated Proteins](#).

## PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.5 mg/mL in sterile PBS.
<b>Shipping</b>	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

$\alpha$ -Fetoprotein is a major plasma protein in the fetus. Its concentration is normally low in the adult except when produced by certain tumors. AFP is produced by the yolk sac and the liver during fetal development. It is thought to be the fetal form of serum albumin. AFP binds to copper, nickel, fatty acids and bilirubin and can found in mono-, di or trimeric forms.