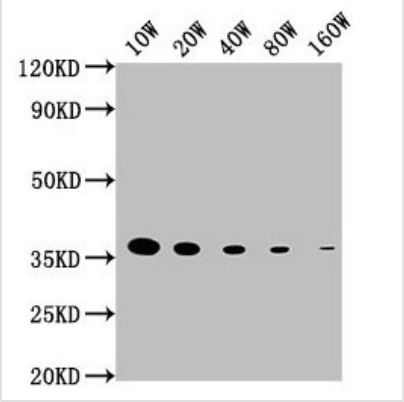
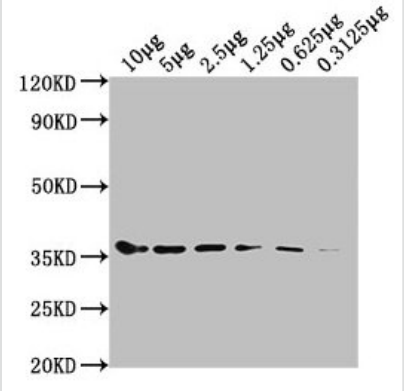
GAPDH Monoclonal Antibody

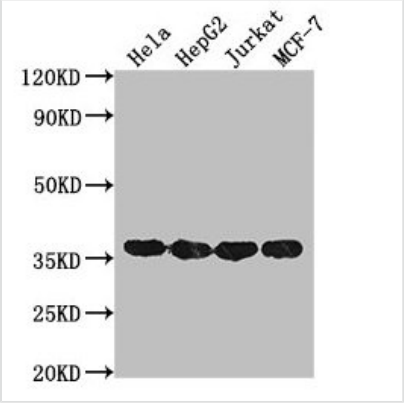
Product Code	CSB-MA000071M0m
Storage	Upon receipt, store at -20°C or -80°C. Avoid repeated freeze.
Immunogen	Recombinant Human GAPDH protein
Raised In	Mouse
Tested Applications	ELISA, WB, IHC, IP, IF, FC; Recommended dilution: WB:1:2000-1:10000, IHC:1:50-1:500, IF:1:50-1:200, IP:1:2000-1:5000
Relevance	Glyceraldehyde 3-phosphate dehydrogenase (GAPDH or G3PDH) is an enzyme of 37kDa that is consisdered as a cellular enzyme involved in glycolysis. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a pleiotropic enzyme that is overexpressed in apoptosis and in several human chronic pathologies. Its role as a mediator for cell death has also been highlighted. At the molecular level, sequential steps lead to nuclear translocation of GAPDH during cell death as follows: first, a catalytic cysteine in GAPDH (C150 in rat GAPDH) is S-nitrosylated by nitric oxide (NO) that is generated from inducible nitric oxide synthase (iNOS) and/or neuronal NOS (nNOS); second, the modified GAPDH becomes capable of binding with Siah1, an E3 ubiquitin ligase, and stabilizes it; third, the GAPDH-Siah protein complex translocates to the nucleus, dependent on Siah1's nuclear localization signal, and degrades Siah1's substrates in the nucleus, which results in cytotoxicity. A recent report suggests that GAPDH may be genetically associated with late-onset of Alzheimer's disease.-deprenyl, which has originally been used as a monoamine oxidase inhibitor for Parkinson's disease, binds to GAPDH and displays neuroprotective actions.
Form	Liquid
Conjugate	Non-conjugated
Storage Buffer	Preservative: 0.03% Proclin 300 Constituents: 50% Glycerol, 0.01M PBS, pH 7.4
Purification Method	>95%, Protein G purified
Isotype	IgG1
Clonality	Monoclonal
Alias	GAPDH; G3PD; GAPD; MGC88685
Product Type	Monoclonal Antibody
Accession NO.	14C2F11
Image	



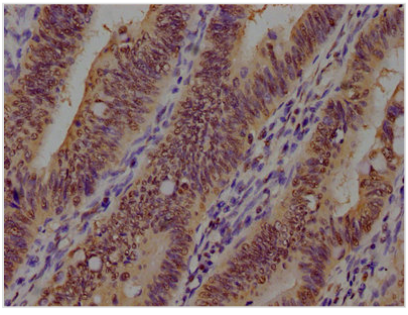
Western Blot
Positive WB detected in: 15µg hela whole cell lysate
GAPDH antibody at 1:10W, 1:20W, 1:40W, 1:80W, 1:160W
Secondary
Goat polyclonal to mouse IgG at 1/50000 dilution
Predicted band size: 36 KDa
Observed band size: 36 KDa
Exposure time: 5min



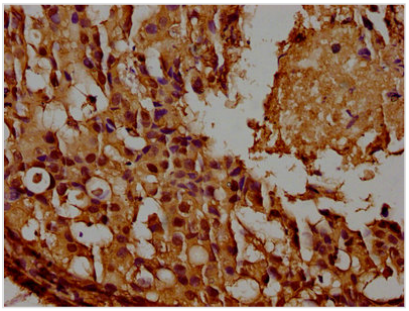
Western Blot
Positive WB detected in: Hela whole cell lysate at 10µg, 5µg, 2.5µg, 1.25µg, 0.625µg, 0.3125µg
All lanes: GAPDH antibody at 1:5000
Secondary
Goat polyclonal to mouse IgG at 1/50000 dilution
Predicted band size: 36 KDa
Observed band size: 36 KDa
Exposure time: 5min



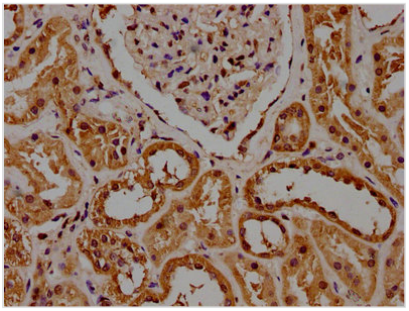
Western Blot
Positive WB detected in: Hela whole cell lysate, HepG2 whole cell lysate, Jurkat whole cell lysate, MCF-7 whole cell lysate
All lanes: GAPDH antibody at 1:2000
Secondary
Goat polyclonal to mouse IgG at 1/50000 dilution
Predicted band size: 36 KDa
Observed band size: 36 KDa
Exposure time: 30s



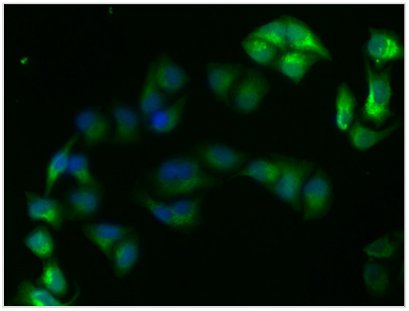
IHC image of CSB-MA000071M0m diluted at 1:100 and staining in paraffin-embedded human colon cancer performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4°C overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.



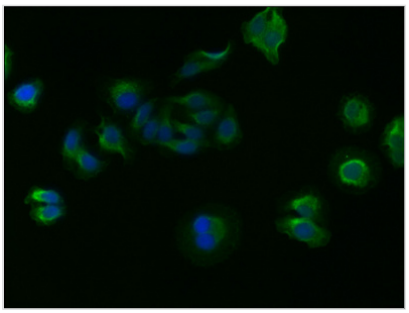
IHC image of CSB-MA000071M0m diluted at 1:100 and staining in paraffin-embedded human breast cancer performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4°C overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.



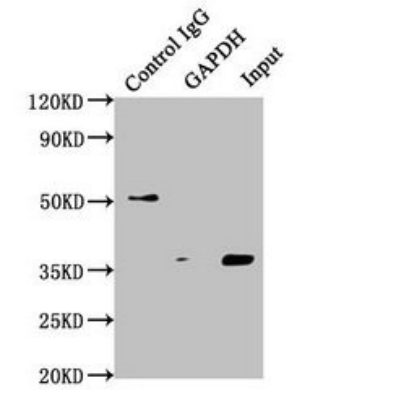
IHC image of CSB-MA000071M0m diluted at 1:100 and staining in paraffin-embedded human kidney tissue performed on a Leica BondTM system. After dewaxing and hydration, antigen retrieval was mediated by high pressure in a citrate buffer (pH 6.0). Section was blocked with 10% normal goat serum 30min at RT. Then primary antibody (1% BSA) was incubated at 4°C overnight. The primary is detected by a biotinylated secondary antibody and visualized using an HRP conjugated SP system.



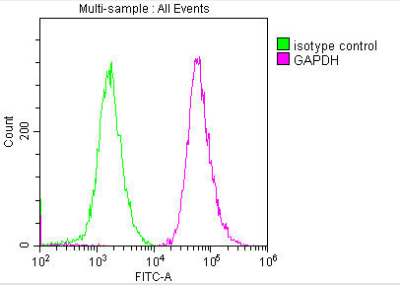
Immunofluorescence staining of Hela cells with CSB-MA000071M0m at 1:220, counter-stained with DAPI. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and blocked in 10% normal Goat Serum. The cells were then incubated with the antibody overnight at 4°C. Nuclear DNA was labeled in blue with DAPI. The secondary antibody was FITC-conjugated AffiniPure Goat Anti-Mouse IgG(H+L).



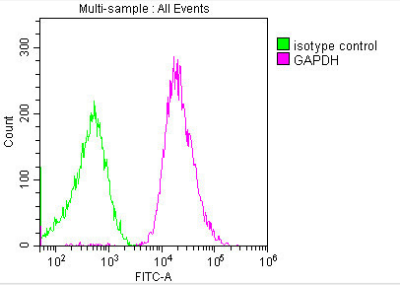
Immunofluorescence staining of HepG2 cells with CSB-MA000071M0m at 1:220, counter-stained with DAPI. The cells were fixed in 4% formaldehyde, permeabilized using 0.2% Triton X-100 and blocked in 10% normal Goat Serum. The cells were then incubated with the antibody overnight at 4°C. Nuclear DNA was labeled in blue with DAPI. The secondary antibody was FITC-conjugated AffiniPure Goat Anti-Mouse IgG(H+L).



Immunoprecipitating GAPDH in Hela whole cell lysate
Lane 1: Mouse control IgG instead of CSB-MA000071M0m in Hela whole cell lysate.
Lane 2: CSB-MA000071M0m (3μl) + Hela whole cell lysate (500μg)
Lane 3: Hela whole cell lysate (20μg)
For western blotting, the blot was detected with CSB-MA000071M0m at 1:5000, and a HRP-conjugated Protein G antibody was used as the secondary antibody at 1:2000



Overlay histogram showing Hela cells stained with CSB-MA000071M0m (red line). The cells were fixed with 70% Ethylalcohol (18h) and then permeabilized with 0.3% Triton X-100 for 2 min. The cells were then incubated in 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (1:200/1*10^{6}cells) for 1 h at 4°C. The secondary antibody used was FITC goat anti-mouse IgG(H+L) at 1/100 dilution for 30min at 4°C. Isotype control antibody (green line) was mouse IgG1 (1:200/1*10^{6}cells) used under the same conditions. Acquisition of >10,000 events was performed.



Overlay histogram showing Jurkat cells stained with CSB-MA000071M0m (red line). The cells were fixed with 70% Ethylalcohol (18h) and then permeabilized with 0.3% Triton X-100 for 2 min. The cells were then incubated in 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (1:200/1*10^{6}cells) for 1 h at 4°C. The secondary antibody used was FITC goat anti-mouse IgG(H+L) at 1/100 dilution for 30min at 4°C. Isotype control antibody (green line) was mouse IgG1 (1:200/1*10^{6}cells) used under the same conditions. Acquisition of >10,000 events was performed.