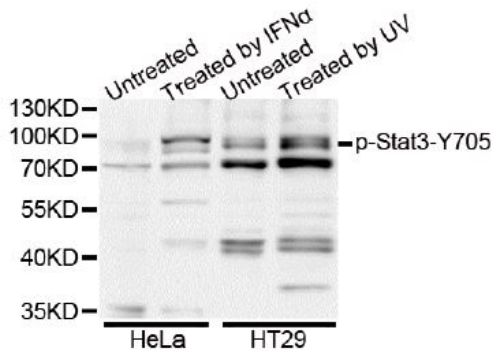
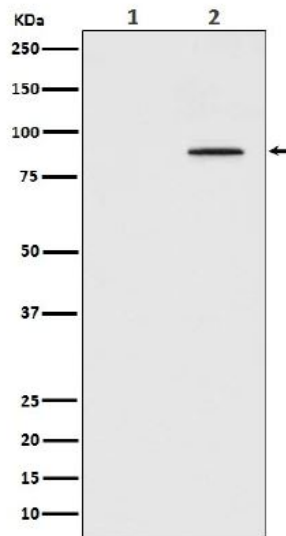


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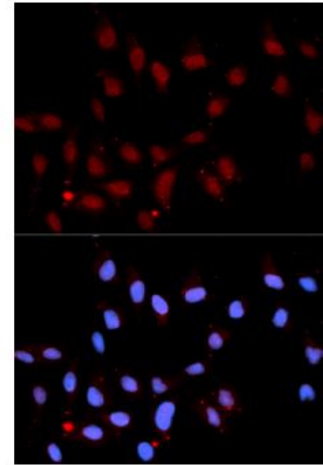
<b>Product name:</b>	Phospho-Stat3 (Y705)
<b>Cat number:</b>	ABP-0070
<b>Conjugate:</b>	Unconjugated
<b>Size:</b>	100 ug
<b>Clone:</b>	Poly
<b>Concentration:</b>	1mg/ml
<b>Host:</b>	Rb
<b>Isotype:</b>	IgG
<b>Reactivity:</b>	Hu, Ms, Rt
<b>Applications:</b>	WB: 1:1000, IHC(P): 1:50, IF: 1:50, IP: 1:10, ICC: 1:50
<b>Molecular Weight:</b>	88 kDa
<b>Purification:</b>	Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Tyr705 of mouse Stat3. Antibodies are purified by protein A and peptide affinity chromatography.
<b>Form:</b>	liquid
<b>Buffer:</b>	Supplied in PBS with 0.02% sodium azide, 50% glycerol, pH7.3.
<b>Storage:</b>	Store at -20°C. Do not aliquot the antibody.
<b>Background:</b>	Stat3 is a key signaling molecule for many cytokines and growth-factor receptors (1) and is required for murine fetal development (2). Additionally, Stat3 is constitutively activated in a number of human tumors (3,4) and possesses oncogenic potential (5) and anti-apoptotic activities (3). Stat3 is activated by phosphorylation at Tyr705, which induces dimerization, nuclear translocation and DNA binding (6,7). Transcriptional activation seems to be regulated by phosphorylation at Ser727 via the MAPK or mTOR pathway (8,9). Stat3 isoform expression appears to reflect biological function: the relative expression levels of Stat3 $\alpha$ (86 kDa) and Stat3 $\beta$ (79 kDa) depend on cell type, ligand exposure or maturation stage of the cells (10). It is notable that Stat3 $\beta$ lacks the serine phosphorylation site within the carboxy-terminal transcriptional activation domain (8). Phospho-Stat3 (Tyr705) Antibody detects endogenous levels of Stat3 only when phosphorylated at Tyr705. The antibody does not cross-react with other Stat proteins when phosphorylated on the corresponding tyrosine residue, but has been shown to cross-react with Phospho-EGFR.



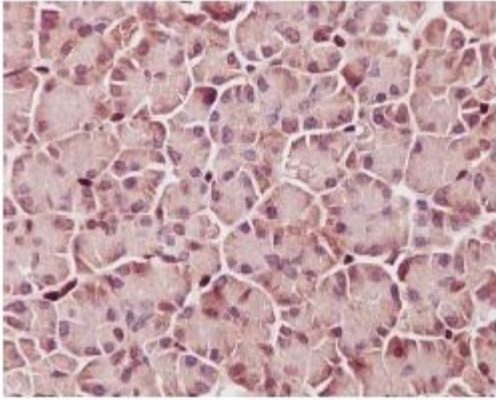
Western blot analysis of extracts of various cell lines, using Phospho-Stat3 (Tyr705) antibody



Western blot analysis of Phospho-STAT3 (Tyr705) expression in (1) HeLa cell lysate; (2) HeLa cell lysate treated with IFN- $\alpha$ . 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 50 $\mu$ g of sample under reducing conditions. 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 rabbit anti-STAT3 monoclonal antibody overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit 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 with Tanon 5200 system. A specific band was detected for STAT3.



Immunofluorescence analysis of U2OS cell using Phospho-Stat3 (Tyr705) antibody. Blue: DAPI for nuclear staining.



Immunohistochemical analysis of paraffin-embedded human pancreas, using Phospho-STAT3 (Y705) Antibody. STAT3 was detected in paraffin-embedded tissue section. Heat mediated antigen retrieval was performed in citrate buffer (pH6, epitope retrieval solution) for 20 mins. The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 $\mu$ g/ml rabbit anti-STAT3 Antibody overnight at 4°C. Biotinylated goat anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Streptavidin-Biotin-Complex (SABC) with DAB as the chromogen.