Purified anti-STAT1 Phospho (Tyr701) Antibody

Catalog# / Size	666401 / 25 µg 666402 / 100 µg
Clone	A17012A
Regulatory Status	RUO
Other Names	Signal Transduced Activator of Transcription 1, Transcription Factor ISGF-3 components p91/p84
Isotype	Mouse IgG1, κ
Description	STAT1, also known as signal transduction and activator of transcription 1, is a ubiquitously expressed cytoplasmic protein and is activated in response to cytokine signaling, including IFN-α, IFN-γ, EGF, PDGF, and IL-6. Upon activation, STAT1 is phosphorylated at Tyrosine 701 (Tyr701) by receptor-associated kinases, including JAK1, JAK2, and TYK2. This results in STAT1 dimerization and subsequent translocation to the nucleus, where it functions as a transcriptional activator. STAT1 is involved in IFN-mediated immune responses, and STAT1-deficient mice are highly sensitive to bacterial and viral infections.

Product Details

Verified Reactivity	Human
Antibody Type	Monoclonal
Host Species	Mouse
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/ml
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	WB - *Quality tested* ICFC, ICC, ChIP - *Verified*
Recommended Usage	Each lot of this antibody is quality control tested by Western blotting. For Western blotting, the suggested use of this reagent is 0.1 - 2.0 µg per ml (1:250-5000 dilution). For intracellular flow cytometric staining, the suggested use of this reagent is ≤0.5 µg per million cells in 100 µl volume. For immunocytochemistry, a range of 5.0 µg/ml is recommended. For ChIP applications, the suggested dilution is 1:50 by volume. It is recommended that the reagent be titrated for optimal performance for each application. For intracellular flow cytometry our True-Nuclear™ Transcription Factor Staining Protocol is recommended.
Application Notes	Clone A17012A recognizes STAT1 phosphorylated at Tyrosine 701 (Tyr701). When using this clone for ICC, we recommend using methanol to permeabilize fixed cells.
RRID	AB_2728499 (BioLegend Cat. No. 666401) AB_2728500 (BioLegend Cat. No. 666402)

Antigen Details

Structure	Two isoforms of STAT1 exist as a result of alternative splicing. Isoform α is a 750 amino acid protein with a predicted molecular weight of 87 kD; the β isoform is a 712 amino acid protein with a predicted molecular weight of 83 kD.

Distribution	Ubiquitous tissue expression, nucleoplasmic-cytosolic distribution
Function	Immune response activation, cell signaling
Interaction	STAT1, STAT2, IRF9, ERBB4, JAK1, JAK2, TYK2, TNK1, SHP2
Biology Area	Cell Biology
Molecular Family	Nuclear Markers, Phospho-Proteins
Antigen References	1. Moretti S, et al. 2017. J. Biol. Chem. 292: 1785. 2. Wei J, et al. 2015. J. Immunol. 195: 2870. 3. Sung PS, et al. 2015. Proc. Natl. Acad. Sci. USA. 112: 10443 4. Ooi EL, et al. 2014. Proc. Natl. Acad. Sci. USA. 111: 1909. 5. Wu TR, et al. 2002. J. Biol. Chem. 277: 47572. 6. Horvath, et al. 1996. J. Virol. 70: 647. 7. Haque SJ, et al. 1995. J. Biol. Chem. 270: 25709.
Gene ID	6772

Related Protocols

BioLegend's Tools for Chromatin Immunoprecipitation (ChIP) Assays - Video

Chromatin Immunoprecipitation (ChIP) Assay Protocol

Immunocytochemistry Staining Protocol

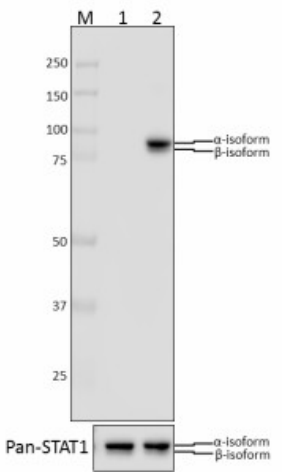
Western Blotting Protocol

Intracellular Staining With True-Phos™ Perm Buffer in Cell Suspensions Protocol

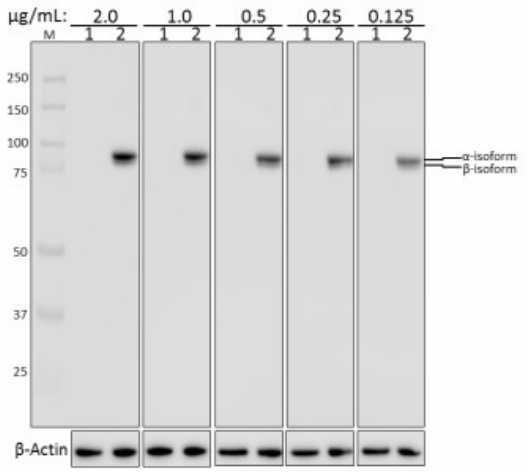
Other Formats

Purified anti-STAT1 Phospho (Tyr701), Go-ChIP-Grade™ Purified anti-STAT1 Phospho (Tyr701), PE anti-STAT1 Phospho (Tyr701), Alexa Fluor® 647 anti-STAT1 Phospho (Tyr701), PerCP/Cyanine5.5 anti-STAT1 Phospho (Tyr701)

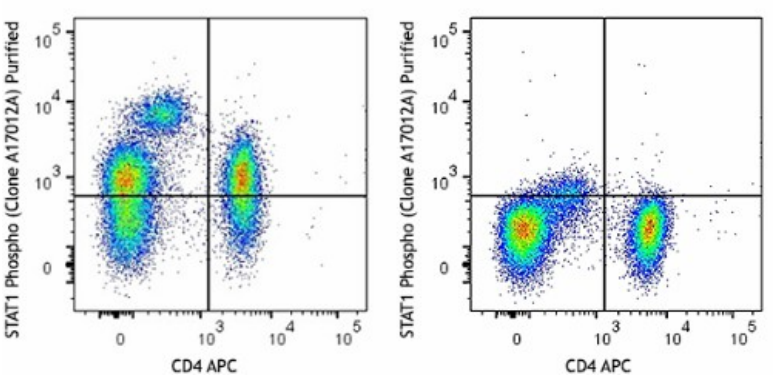
Product Data



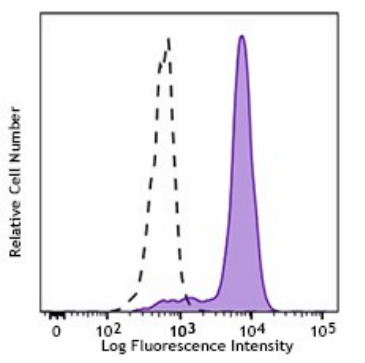
Total cell lysates (15 µg protein) from serum-starved HeLa S3 cells treated without (Lane 1) or with (Lane 2) 10 ng/mL human IFN-α2 (Cat. No. 592702) for 10 minutes were resolved by 8% Bis-Tris gel electrophoresis, transferred to nitrocellulose, and probed with 2 µg/mL (1:250 dilution) purified anti-STAT1 Phospho (Tyr701) antibody (clone A17012A). Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse-IgG (Cat. No. 405301) at a 1:3000 dilution. Equal STAT1 loading was confirmed using a pan anti-STAT1 antibody that recognizes both STAT1 α (87 kD) and β (83 kD) isoforms.



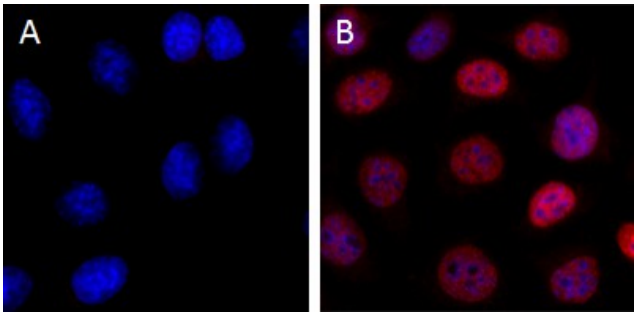
Total cell lysates (15 µg protein) from serum-starved HeLa S3 cells treated without (Lane 1) or with (Lane 2) 10 ng/mL human IFN-α2 (Cat. No. 592702) for 10 minutes were resolved by 8% Bis-Tris gel electrophoresis, transferred to nitrocellulose, and probed with the indicated concentration of purified anti-STAT1 Phospho (Tyr701) antibody (clone A17012A). Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse-IgG (Cat. No. 405301) at a 1:3000 dilution. Equal protein loading was confirmed using Direct-Blot™ HRP anti-β-Actin antibody (Cat. No. 643807) used at a 1:5000 dilution.



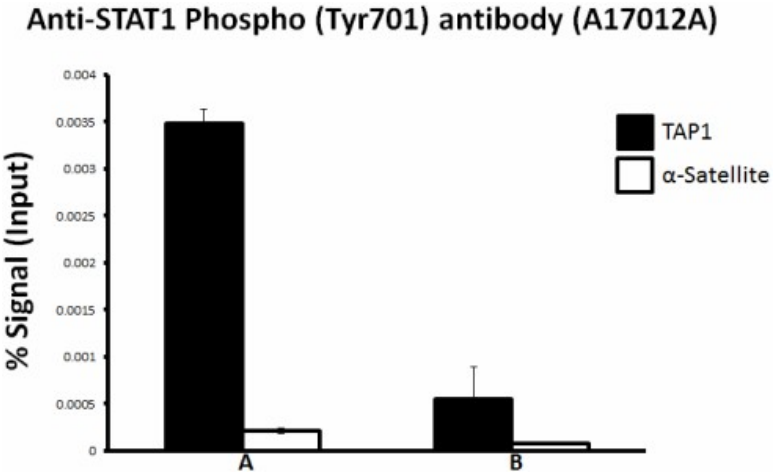
Human peripheral blood mononuclear cells were treated with (left), or without (right) Recombinant Human Interferon-γ (Cat No. 570204) for 15 minutes, fixed with Fixation Buffer (Cat. No. 420801), permeabilized with True-Phos™ Perm Buffer (Cat. No. 425401) then stained with CD4 (clone A161A1) APC and purified anti-STAT1 Phospho (Tyr701) (clone A17012A), followed by anti-mouse IgG PE. Data was gated on lymphocyte and monocyte populations.



Human peripheral blood monocytes were treated with (filled histogram), or without (open histogram) Recombinant Human Interferon-γ (Cat. No. 570204) for 15 minutes, fixed with Fixation Buffer (Cat. No. 420801), permeabilized with True-Phos™ Perm Buffer (Cat. No. 425401) then stained with purified anti-STAT1 Phospho (Tyr701) (clone A17012A), followed by anti-mouse IgG PE.



Serum starved HeLa cells were untreated (panel A) or stimulated with 100 ng/mL IFN-α2 (Cat. No. 592702, panel B) for 20 minutes, fixed with 4% paraformaldehyde for 10 minutes, permeabilized with ice-cold methanol for 10 minutes, and blocked with 5% FBS for 60 minutes. Cells were then intracellularly stained with a 1:100 dilution (5 µg/mL) of purified anti-STAT1 Phospho (Tyr701) Antibody, clone A17012A, for two hours at room temperature, followed by incubation with Alexa Fluor® 594 goat anti-mouse IgG Antibody(Cat. No. 405326) at 2.0 µg/mL. Nuclei were counterstained with DAPI, and the image was captured with a 60X objective.



Chromatin Immunoprecipitations (ChIP) were performed with cross-linked chromatin samples from 4 X 10^6 of HT1080 cells treated with IFNγ for 30 minutes with either A)1:50 dilution of Go-ChIP-Grade™ Purified anti-STAT1 Phospho (Tyr701) (Clone A17012A) or B) equal amount of Purified Mouse IgG1, κ Isotype Control Antibody (Clone MG1-45, Cat. No. 401401) by using Go-ChIP-Grade™ Protein G Enzymatic Kit (Cat. No. 699904). The enriched DNA was purified and quantified by real-time qPCR using primers targeting human TAP1 gene region and α-Satellite repeats. The amount of immunoprecipitated DNA in each sample is represented as signal relative to total amount of input chromatin.

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