bs-4076R **[Primary Antibody]**

phospho-E2F1 (Ser364) Rabbit pAb

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DATASHEET

Host: Rabbit **Isotype:** IgG

Clonality: Polyclonal

GeneID: 1869 **SWISS:** Q01094

Target: E2F1 (Ser364)

Immunogen: KLH conjugated Synthesised phosphopeptide derived from human E2F1 around the phosphorylation site of Ser364: MG(p-S)LR.

Purification: affinity purified by Protein A

Concentration: 1mg/ml

Storage: 0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol.
Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.

Background: E2F's are DNA binding proteins, which associate with negative regulators, such as the retinoblastoma p107 protein, resulting in an altered rate of gene transcription. The E2F proteins contain several evolutionally conserved domains found in most members of the family. These domains include a DNA binding domain, a dimerization domain which determines interaction with the differentiation regulated transcription factor proteins (DP), a transactivation domain enriched in acidic amino acids, and a tumor suppressor protein association domain which is embedded within the transactivation domain. This protein and another 2 members, E2F2 and E2F3, have an additional cyclin binding domain. E2F1 is proposed to be involved in several cellular processes that range from tumor suppressor, cell progression and oncogenesis. E2F1 overexpression can also drive cells into apoptosis.

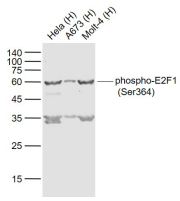
Applications: **WB** (1:500-2000)
IHC-P (1:100-500)
IHC-F (1:100-500)
IF (1:100-500)
Flow-Cyt (1ug/Test)
ICC/IF (1:100)

Reactivity: Human, Rat (predicted: Mouse)

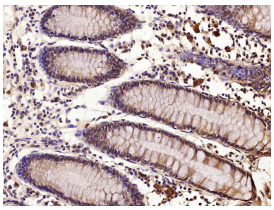
Predicted MW.: 48 kDa

Subcellular Location: Nucleus

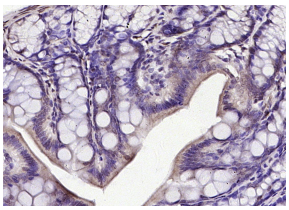
VALIDATION IMAGES



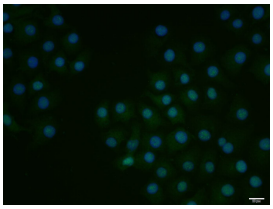
Sample: Lane 1: Hela (Human) Cell Lysate at 30 ug Lane 2: A673 (Human) Cell Lysate at 30 ug Lane 3: Molt-4 (Human) Cell Lysate at 30 ug Primary: Anti-phospho-E2F1 (Ser364) (bs-4076R) at 1/1000 dilution Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution Predicted band size: 55-60 kD Observed band size: 60 kD

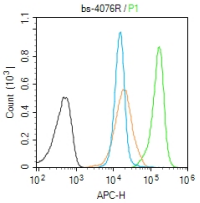


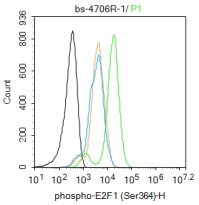
Paraformaldehyde-fixed, paraffin embedded (Human colon carcinoma); Antigen retrieval by microwave in sodium citrate buffer (pH6.0) ; Block endogenous peroxidase by 3% hydrogen peroxide for 30 minutes; Blocking buffer (3% BSA) at RT for 30min; Antibody incubation with (phospho-E2F1 (Ser364)) Polyclonal Antibody, Unconjugated (bs-4076R) at 1:400 overnight at 4°C, followed by conjugation to the secondary antibody (labeled with HRP)and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat colon); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-E2F1 (Ser364)) Polyclonal Antibody, Unconjugated (bs-4076R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.







HepG2 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (phospho-E2F1 (Ser364)) polyclonal Antibody, Unconjugated (bs-4076R) 1:100, 90 minutes at 37°C; followed by a conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.

Blank control (Black line):Molt4 (Black). Primary Antibody (green line): Rabbit Anti-phospho-E2F1 (Ser364) antibody (bs-4076R) Dilution: 1µg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647 Dilution: 1µg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at room temperature. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

Blank control:HepG2. Primary Antibody (green line): Rabbit Anti-phospho-E2F1 (Ser364) antibody (bs-4076R) Dilution: 1ug/Test; Secondary Antibody : Goat anti-rabbit IgG-FITC Dilution: 0.5ug/Test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 0.1% PBST for 20 min at room temperature.The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.