## Anti-Phospho-ATR (T1989) Antibody [PS01-18] HA721190

Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell

Molecular Wt: Predicted band size: 301 kDa

Clone number: PS01-18

Description: Serine/threonine protein kinase which activates checkpoint signaling upon genotoxic

stresses such as ionizing radiation (IR), ultraviolet light (UV), or DNA replication stalling, thereby acting as a DNA damage sensor. Recognizes the substrate consensus sequence [ST]-Q. Phosphorylates BRCA1, CHEK1, MCM2, RAD17, RPA2, SMC1 and p53/TP53, which collectively inhibit DNA replication and mitosis and promote DNA repair, recombination and apoptosis. Phosphorylates 'Ser-139' of histone variant H2AX at sites of DNA damage, thereby regulating DNA damage response mechanism. Required for FANCD2 ubiquitination. Critical for maintenance of fragile site stability and efficient regulation of centrosome duplication. Positively regulates the restart of stalled replication forks following activation by

the KHDC3L-OOEP scaffold complex.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Thr1989 of human ATR.

Positive control: HeLa cell lysate, HeLa treated with Hydroxyurea cell lysate, HeLa treated with 4mM

Hydroxyurea for 20 hours.

**Subcellular location:** Chromosome, Nucleus.

Database links: SwissProt: Q13535 Human

**Recommended Dilutions:** 

**WB** 1:1,000 **IF-Cell** 1:200

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

**Storage Instruction:** Store at  $+4^{\circ}$ C after thawing. Aliquot store at  $-20^{\circ}$ C. Avoid repeated freeze / thaw cycles.

**Purity:** Protein A affinity purified.

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## **Images**

**Fig1:** Western blot analysis of Phospho-ATR (T1989) on different lysates with Rabbit anti-Phospho-ATR (T1989) antibody (HA721190) at 1/1,000 dilution.

Lane 1: HeLa cell lysate

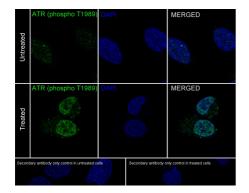
Lane 2: HeLa treated with Hydroxyurea cell lysate

Lysates/proteins at 20 µg/Lane.

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721190) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.



**Fig2:** Immunocytochemistry analysis of HeLa cells treated with or without 4mM Hydroxyurea for 20 hours labeling Phospho-ATR (T1989) with Rabbit anti-Phospho-ATR (T1989) antibody (HA721190) at 1/200 dilution. Image shown an increased nuclear staining upon Hydroxyurea treatment.

Cells were fixed in 4% paraformaldehyde for 20 minutes at 37  $^{\circ}$ C, permeabilized with 0.1% Triton X-100 in PBS permeabilization for 5 minutes, and then blocked with 2% negative goat serum for 60 minutes at room temperature. Cells were then incubated with Rabbit anti-Phospho-ATR (T1989) antibody (HA721190) at 1/200 dilution in 1% BSA overnight at 4  $^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor \*\* 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

## **Background References**

- 1. Ma M et al. Activation of ATR-related protein kinase upon DNA damage recognition. Curr Genet. 2020 Apr
- 2. Tiernan H et al. ATR-FTIR spectroscopy and spectroscopic imaging for the analysis of biopharmaceuticals. Spectrochim Acta A Mol Biomol Spectrosc. 2020 Nov