

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Chk2 when phosphorylated at T68.
Source	Polyclonal Rabbit IgG
Purification	Antigen Affinity-purified
Immunogen	Phosphopeptide containing human Chk2 T68 site
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p> <p>Detection of Human Phospho-Chk2 (T68) by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line untreated (-) or treated (+) with 1 µM camptothecin (CPT) for 1 hour. PVDF membrane was probed with 0.5 µg/mL Rabbit Anti-Human Phospho-Chk2 (T68) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1626) followed by HRP-conjugated Anti-Rabbit IgG Secondary Antibody (Catalog # HAF008). A specific band for Phospho-Chk2 (T68) was detected at approximately 64 kDa (as indicated). The phospho-specificity of this antibody was supported by decreased labeling following treatment with 600 U λ-phosphatase (λ-PPase) for 60 minutes. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p> <p>Phospho-Chk2 (T68) in HepG2 Human Cell Line. Phospho-Chk2 (T68) was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line treated with 1 µM camptothecin using Rabbit Anti-Human Phospho-Chk2 (T68) Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1626) at 1 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rabbit IgG Secondary Antibody (red; Catalog # NL004) and counterstained with DAPI (blue). Specific staining was localized to nuclei in treated cells. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> ● 12 months from date of receipt, -20 to -70 °C as supplied. ● 1 month, 2 to 8 °C under sterile conditions after reconstitution. ● 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

ATM phosphorylates the Checkpoint kinase on T68 in response to genotoxic insults. Phosphorylation of T68 promotes the formation of Chk2 oligomers. Oligomerization is a critical event in the activation of the Chk2 kinase as it is required for phosphorylation of additional Chk2 residues that lead to full activation and signaling to downstream effectors.