



cell signaling solutions

Certificate of Analysis

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Anti-phospho-BRCA1 (Ser1423)

(rabbit polyclonal IgG)

Catalog # 07-635

Lot # 27090

Immunogen: KLH-conjugated, synthetic peptide containing the sequence ...GpSQP... in which pS corresponds to phosphoserine 1423 of human BRCA1. Accession # NM_007294.

Specificity: Recognizes phospho-BRCA1 (Ser1423), Mr 200-220kDa. Specificity was demonstrated by blocking the antibody with the immunizing peptide and treating the blot with λ -phosphatase.

Species Cross-reactivity: Human.

Formulation: 200 μ g of protein A purified rabbit IgG in 200 μ l of 0.1M Tris glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

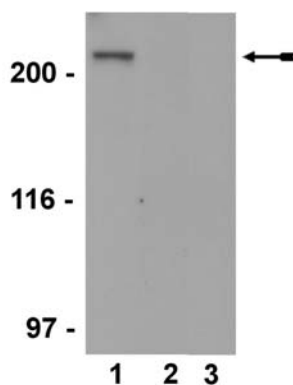
FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 1-2 μ g/ml of this lot detected phospho-BRCA1 (Ser1423) in HeLa nuclear extract.

Peptide Inhibition Analysis: 5 μ M of the immunizing peptide abolished detection of phospho-BRCA1 (Ser1423) in immunoblot analysis of HeLa nuclear extract.

Included Positive Antigen Control: Catalog # 12-309, HeLa nuclear extract. **Add an equal volume of Laemmli reducing sample buffer to 5-10 μ l of extract and boil for 5 minutes to reduce the preparation.** Load 20 μ g of reduced lysate per lane for minigels.



Immunoblot Analysis

HeLa nuclear extract (Lanes 1 and 3) and HeLa nuclear extract after λ -phosphatase treatment (Lane 2) was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho-BRCA1 (Ser1423) (1 μ g/ml; Lanes 1 and 2) or anti-phospho-BRCA1 (Ser1423) pre-incubated with 5 μ M of immunizing peptide (Lane 3). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates BRCA1.

General References:

1. Ruffner, H., and Verma, I.M., *Proc. Natl. Acad. Sci. USA* **94**: 7138-7143, 1997.
2. Xu, B., et al., *Mol. Cell Biol.* **21**: 3445-3450, 2001.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a HeLa nuclear extract sample (using a modified protocol of Dignam, *et al.*, Nucleic Acids Res. **22**: 1475, 1983) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 5% nonfat dry milk (Catalog # 20-200) in TBS with 0.05% Tween[®]-20 (TBST-MLK) for 2 hours at room temperature with constant agitation.
3. Incubate the nitrocellulose with **1-2µg/ml of anti-phospho-BRCA1 (Ser1423)**, diluted in freshly prepared TBST-MLK for 2 hours with agitation at room temperature.
4. Wash the nitrocellulose three times with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:3000 dilution was used) in TBST-MLK for 1.5 hours with agitation at room temperature.
6. Rinse the nitrocellulose twice with water.
7. Wash the nitrocellulose in TBS-0.05% Tween[®]-20 for 3-5 minutes.
8. Wash the nitrocellulose three times with water.
9. Use detection method of choice (enhanced chemiluminescence was used).