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Monoclonal Antibody to BRCA1 / RNF53 - Purified

Alternate names:	Breast cancer type 1 susceptibility protein, RING finger protein 53
Catalog No.:	AM39123PU-N
Quantity:	0.1 mg
Concentration:	1.0 mg/ml (lot-specific)
Background:	Breast cancer type 1 susceptibility protein (BCRA1; RING finger protein 53) functions as a tumor suppressor. BRCA1 is an important member of the DNA repair pathway. After DNA damage, BRCA1 is phosphorylated on multiple serine residues by the ATR kinase or Chk2, and is localized to lesion sites with PCNA. BRCA1 interacts with a wide range of proteins including Rad50, NBS1, and Mre11, c-Abl tyrosine kinase, and γH2A.X, involved in the detection of damaged DNA and activation of appropriate repair pathways. BRCA1 may also respond to DNA damage by promoting gene expression through association with transcriptional proteins and RNA polymerase II, and may regulate the p53 pathway by this mechanism. Previous studies have indicated that mutations in the BRCA1 gene may contribute to the development of breast and ovarian cancer.
Uniprot ID:	P38398
NCBI:	9606
GeneID:	672
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM10P (for use in human samples), AM03095PU-N
Clone:	MS13
Immunogen:	Recombinant protein corresponding to human BRCA1
Format:	**State:** Liquid purified IgG1 fraction **Purification:** Protein G chromatography **Buffer System:** 0.1 M Tris-Glycine (pH 7.4), 150 mM NaCl with 0.05% sodium azide
Applications:	**Western Blot**: 0.5 µg/mL of this antibody detected BRCA1 on 10 µg of HeLa nuclear extract. **Immunohistochemistry**: A 1:50 dilution from a representative lot detected BRCA1 in human breast cancer and human ovarian cancer tissues. **Immunocytochemistry**: A 1:500 dilution from a representative lot detected BRCA1 in HeLa and A431 cells. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Molecular Weight:	~220 kDa observed. The calculated molecular weight of this protein is 208 kDa, but can be observed at ~220 kDa due to high glycosylation.

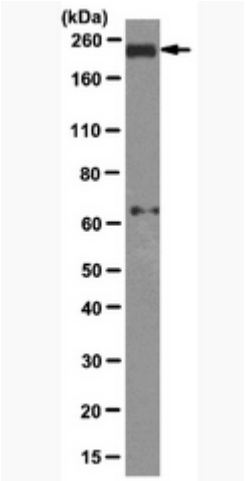
Specificity:
The antibody reacts with Human BRCA1.
Other species not tested.

Storage:
Store undiluted at 2-8°C.
Shelf life: One year from despatch.

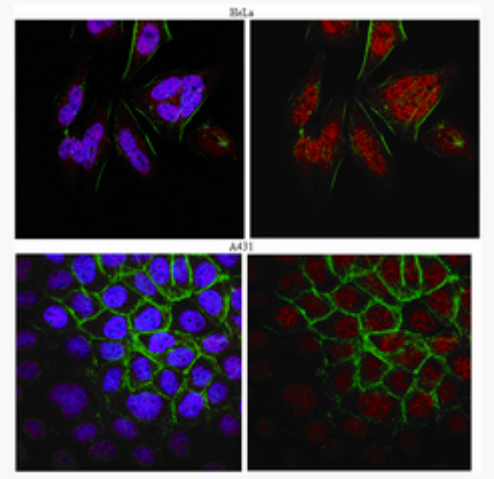
General Readings:
1. Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science. 1999 Nov 5;286(5442):1162-6. PubMed PMID: 10550055.
2. Roy R, Chun J, Powell SN. BRCA1 and BRCA2: different roles in a common pathway of genome protection. Nat Rev Cancer. 2011 Dec 23;12(1):68-78. doi: 10.1038/nrc3181. PubMed PMID: 22193408.

Protocols:
Western Blotting
1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA or milk with 0.05% Tween®-20 surfactant for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of the primary antibody, diluted in freshly prepared 5% BSA or milk for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Visualize with enhanced chemiluminescence (ECL) method of choice.

Pictures:
Western Blotting:
HeLa nuclear extract was probed with Cat. No. AM39123PU-N, Anti-BRCA1, clone MS13 (0.5 µg/mL). Proteins were visualized using a Goat Anti-Mouse IgG secondary antibody conjugated to HRP and a chemiluminescence detection system. The arrow indicates BRCA1 (~220 kDa).
Please Note: An uncharacterized band appears at ~65 kDa in some lysates.



Immunocytochemistry:
Confocal fluorescent analysis of A431 and HeLa cells using Anti-BRCA1 Donkey Cat. No. AM39123PU-N with Anti-Mouse IgG Cy3 (Red). Actin filaments have been labeled with Alexa Fluor® 488 dye-Phalloidin (Green). Nucleus is stained with DAPI (Blue). This antibody positively stains the nucleus with some staining on cytoplasm.



Immunohistochemistry:
Formalin Fixed Paraffin Embedded (FFPE) human breast cancer (Fig. 1 & 2) and human ovarian cancer (Fig. 3) tissues were prepared using heat-induced epitope retrieval (HIER). Immunostaining was performed using a 1:50 dilution of Cat. No. AM39123PU-N, Anti-BRCA1, clone MS13. Reactivity was detected using an Anti-Mouse secondary antibody and HRP-DAB. Nuclear and cytoplasmic staining was observed in human breast and ovarian cancer cells.