

ORDERING INFORMATION

Catalog Number: MAB2476

Clone: 234403

Lot Number: UFN01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

Reconstitution: sterile PBS

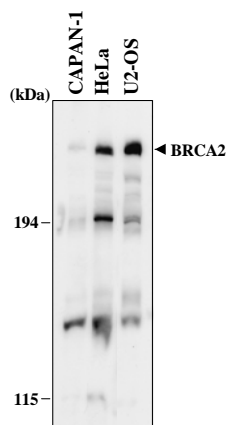
Specificity: human BRCA2

Immunogen: *E. coli*-derived rhBRCA2

Ig class: mouse IgG₁

Recommended Applications:

Western blot
Immunohistochemistry



Detection of human BRCA2 with

MAB2476. Extracts were prepared from human CAPAN-1 (contains mutant BRCA2 with carboxyl-terminal truncation), HeLa, or U2-OS cells. Equal amounts of cellular extracts were resolved by SDS-PAGE, transferred to a PVDF membrane, and immunoblotted with 0.2 µg/mL monoclonal anti-human BRCA2 antibody.

Background

BRCA2 is a nuclear protein that acts as a tumor suppressor. Germline mutation of BRCA2 accounts for many cases of familial breast and ovarian cancer. At the cellular level, BRCA2 is involved in homologous recombination (HR), a form of double-strand break repair. BRCA2 interacts with the Rad51 recombinase, another protein critical for HR, to preserve chromosomal integrity.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, *E. coli*-derived, recombinant human BRCA2 (rhBRCA2; aa 1 - 200). The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS.

Storage

Lyophilized antibodies are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

The antibody was selected for its ability to detect human BRCA2 in Western blots and immunohistochemistry experiments.

Applications

Western Blot - An antibody concentration of 0.1 - 0.5 µg/mL is recommended.

Immunohistochemistry - This antibody was used at a concentration of 25 µg/mL with appropriate secondary reagents to detect BRCA2 in paraffin-embedded human breast cancer tissue sections. For chromogenic detection of labeling, the use of R&D Systems' Cell and Tissue Staining Kits (CTS Series) is recommended.

Optimal dilutions should be determined by each laboratory for each application.

Protocol for Immunoblotting:

Blotting Buffer	Blocking Solution
25 mM Tris, pH 7.5	5% nonfat dry milk
0.15 M NaCl	in Blotting Buffer
0.05% Tween 20	pH to 7.5

1. Transfer the electrophoresed proteins onto a PVDF membrane and incubate the membrane for 1 hour at room temperature in Blocking Solution.
2. Incubate the membrane for 2 hours at room temperature or overnight at 2 - 8° C in Blocking Solution containing 0.1 - 0.5 µg/mL anti-human BRCA2.
3. Wash the membrane at room temperature for 30 minutes with 3 or more changes of Blotting Buffer. Changing the membrane containers often reduces background.
4. Incubate the membrane at room temperature for 1 hour in Blocking Solution containing a 1:1,000 dilution of anti-mouse IgG-HRP (R&D Systems, Catalog # HAF007).
5. Wash the membrane for 30 minutes with 3 or more changes of Blotting Buffer.
6. Detect with WesternGlo™ Chemiluminescent Detection Substrate (R&D Systems, Catalog #AR004).

Made under one or more of the following US Patents: 5,837,492; 6,124,104.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

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Cell Lysates for Western Blotting: To prepare total cell lysates, solubilize cells in 2X SDS gel sample buffer (20 mM dithiothreitol, 6% SDS, 0.25 M Tris, pH 6.8, 10% glycerol, and bromophenyl blue) and sonicate with a probe sonicator using 3 - 4 bursts of 5 - 10 seconds each. Heat extracts in a boiling water bath for 5 minutes and load onto polyacrylamide gels. Samples may be diluted with 1X SDS sample buffer to the desired concentration.