TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

Anti-phosphorylated Histone H2AX (γ-H2AX) Polyclonal Antibody

Catalog #: 4411-PC-020 **Size:** 20 µl

Background: Histone H2AX is a 14 kDa ubiquitous member of the H2A histone family that contains an evolutionarily conserved SQ motif at the C-terminus in eukaryotes. Serine 139 within this motif becomes rapidly phosphorylated to yield a form known as γ -H2AX in response to double-strand DNA damage and apoptosis. Phosphorylation reaches half its maximum between 1-3 minutes after DNA damage occurs, and hundreds to several thousand molecules of γ-H2AX are present per double-strand break. This antibody is unique in only detecting double-strand DNA breaks.

Physical State: Rabbit serum containing polyclonal antibody raised against synthetic phosphorylated peptide. Provided at 8 µg/µl in phosphate buffered saline with 0.01% sodium azide.

Specificity: Recognizes mammalian, yeast, *D. melanogaster*, and *X. laevis* γ-H2AX.

Storage: Freeze in working aliquots at -20°C to avoid repeated freeze-thawing.

Applications: Suitable for Western blotting and immunocytochemistry. For Western blot analysis, a starting dilution of 1:1000 is recommended, whereas for IC, a starting dilution of 1:100 is recommended. Empirical determination of antibody concentration is required for optimal results.

References:

1. Mahadevaiah, S.K., J.M. Turner, F. Baudat, E.P. Rogakou, P. de Boer, J. Blanco-Rodriguez, M. Jasin, S. Keeney, W.M. Bonner, P.S. Burgoyne. 2001. Recombinational DNA double-strand breaks in mice precede synapsis. Nature Gen **27**:271-6.
2. Rogakou, E.P., W.Nieves-Neira, C.Boon, Y.Pommier, and W.M.Bonner. 2000. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. J Biol Chem **275**:9390-5
3. Rogakou, E.P., C. Boon, C. Redon, W.M. Bonner. 1999. Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol **146**:905-16.
4. Rogakou, E.P., D.R. Pilch, A.H. Orr, V.S. Ivanova, W.M. Bonner. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem. **273**:5858-68.

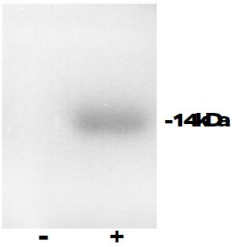

Fig. 1. Immunoblot of SDS-extracts from Jurkat cells treated with and with-out 120 µM etoposide for 4 hours. Samples were electrophoresed on an 18% Tris-Glycine gel and transferred onto a PVDF membrane. γ-H2AX was detected with anti-phosphorylated Histone H2AX antibody followed by anti-rabbit conjugated to horseradish peroxidase and chemiluminescence.

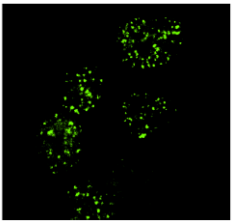

Fig 2. Human cancer (NCI/ADR) cells were irradiated with 2 Gy to introduce dsDNA breaks. After fixation and permeabilization, cells were labeled with anti-phosphorylated histone H2AX followed by an anti-rabbit fluorescein conjugate. Photo courtesy of Dr. E. Rogakou, NCI, NIH, Bethesda, MD.

Patent Pending 09/351,721

 E8/2/10v1

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Procedure for Immunoblotting using Peroxidase Detection:

Transfer the electrophoresed proteins to nitrocellulose or PVDF membrane by Western transfer. Incubate the membrane for 30 minutes at room temperature in 2% (v/v) Bovine or Horse serum in PBS. The use of milk as a blocking agent is not recommended.

Incubate the membrane for 1 hour at room temperature (or overnight at 4°C) in Trevigen's anti-phosphorylated histone H2AX antibody diluted 1:1000 in PBS containing 2% (v/v) serum and 0.05% Tween 20. Empirical determination of primary antibody concentration will be required for optimal results.

Wash the membrane at room temperature for at least 15 minutes with 3 changes of PBS, 0.05% Tween 20. Changes in solution containers often reduces background.

Incubate the membrane at room temperature for 1 hour in PBS containing a dilution of anti-rabbit antibody conjugated to Horseradish peroxidase, 2% serum and 0.05% Tween 20. Empirical determination of secondary antibody concentration will be required for optimal results.

Wash the membrane for at least 15 minutes with 3 changes of PBS, 0.05% Tween 20.

Develop peroxidase reaction using an HRP membrane solution or chemiluminescence reagents.

Related Products:

Catalog #	Description	Size
6370-MC-100	Anti-human/murine-Cytochrome C	100 µg
6380-MC-100	Anti-human/murine-Holocytochrome C	100 µg
2291-MC-100	Anti-human-Bcl-2 mAb (clone YTH-8C8)	100 µg
2281-MC-100	Anti-human-Bax mAb (clone YTH-6A7)	100 µg
6361-PC-100	Anti-human/mouse-PBR polyclonal	100 µl
4335-MC-100	Anti-PAR polymer mAb (10HA)	100 µl
4336-BPC-100	Anti- PAR polymer polyclonal	100 µl
4338-MC-50	Anti-human/murine-PARP mAb (clone C2-10)	50 µg