

# TREVIGEN® Product Data

For Research Use Only. Not For Use In Diagnostic Procedures

## Anti-phosphorylated Histone H2AX ( $\gamma$ -H2AX) Polyclonal Antibody

Catalog #: 4411-PC-100

Size: 100  $\mu$ l

**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  $\gamma$ -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  $\gamma$ -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  $\mu$ g/ $\mu$ l in phosphate buffered saline with 0.01% sodium azide.

**Specificity:** Recognizes mammalian, yeast, *D. melanogaster*, and *X. laevis*  $\gamma$ -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. Tumer, 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.

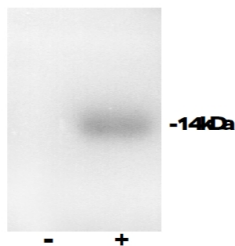


Fig. 1. Immunoblot of SDS-extracts from Jurkat cells treated with and without 120  $\mu$ M etoposide for 4 hours. Samples were electrophoresed on an 18% Tris-Glycine gel and transferred onto a PVDF membrane.  $\gamma$ -H2AX was detected with anti-phosphorylated Histone H2AX antibody followed by anti-rabbit conjugated to horseradish peroxidase and chemiluminescence.

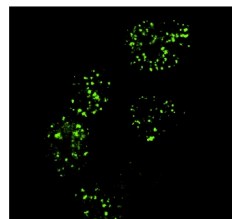


Fig 2. Human cancer (NCI/ADR) cells were irradiated with 2 Gy to introduce dsDNA breaks. After fixation and permeabilization, cells were labeled with anti-phosphorylated histone H2AX followed by an anti-rabbit fluorescein conjugate. Photo courtesy of Dr. E. Rogakou, NCI, NIH, Bethesda, MD.

Patent Pending 09/351,721

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## TREVIGEN®

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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 Trevigen's Blue Membrane Solution (Cat# 4857-20-13) or PeroxyGlow chemiluminescence reagents (Cat#s 4855-20-13 and 4855-20-14).

## Related Products:

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

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Polyclonal Antibody

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Storage: -20 °C

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