Phospho-Histone H2A.X (Ser139) Monoclonal Antibody (CR55T33), eFluor™ 660, eBioscience™

Product Details	
Size	100 Tests
Species Reactivity	Human, Mouse
Published Species	Human
Host/Isotype	Mouse / IgG1, kappa
Recommended Isotype Control	Mouse IgG1 kappa Isotype Control (P3.6.2.8.1), eFluor™ 660, eBioscience™
Class	Monoclonal
Type	Antibody
Clone	CR55T33
Conjugate	eFluor™ 660
Excitation/Emission Max	651/668 nm
Form	Liquid
Concentration	5 µL/Test
Purification	Affinity chromatography
Storage buffer	PBS, pH 7.2, with 0.2% BSA
Contains	0.09% sodium azide
Storage conditions	4° C, store in dark, DO NOT FREEZE!
RRID	AB_2574399

Applications	Tested Dilution	Publications
Western Blot (WB)	1:400	2 Publications
Immunohistochemistry (IHC)	-	1 Publication
Immunocytochemistry (ICC/IF)	1:50	-
Flow Cytometry (Flow)	5 µL (0.125 µg)/test	3 Publications

Product Specific Information

Description: The CR55T33 monoclonal antibody recognizes phosphorylated serine 139 of human and mouse H2AX. H2AX is a member of the H2A histone family that complex with DNA and other histones to form the repeating nucleosome units characteristic of eukaryotic chromatin. Nucleosomes consist of approximately 147 base pairs of DNA wrapped around an octamer of histones composed of two each of the four histone proteins: H2A, H2B, H3 and H4. After induction of DNA damage such as double-strand breaks by irradiation, genotoxic stresses, replication errors or gene recombination, PI3K-like kinases (e.g., ataxia telangiectasia mutated (ATM), ataxia telangiectasia Rad-3-related (ATR), and DNA-dependent protein kinase (DNA-PK)) are activated to phosphorylate serine 139 in H2AX. This early phosphorylation event plays a critical role in recruiting proteins involved in DNA repair.

The monoclonal antibody CR55T33 recognizes a single band of approximately 15 kDa on reduced cell lysates from Jurkat cells stimulated with etoposide.

Applications Reported: This CR55T33 antibody has been reported for use in intracellular staining followed by flow cytometric analysis, and immunocytochemistry.

Applications Tested: This CR55T33 antibody has been pre-titrated and tested by intracellular staining followed by flow cytometric analysis of treated human peripheral blood cells using the Foxp3/Transcirption Factor Buffer Set (Product # 00-5523-00) and protocol. This can be used at 5 µL (0.125 µg) per test. A test is defined as the amount (µg) of antibody that will stain a cell sample in a final volume of 100 µL. Cell number should be determined empirically but can range from 10^5 to 10^8 cells/test. The CR55T33 antibody has also been tested by immunocytochemistry of methanol-fixed human cells and can be used at less than or equal to 10 µg/mL. It is recommended that the antibody be carefully titrated for optimal performance in the assay of interest.

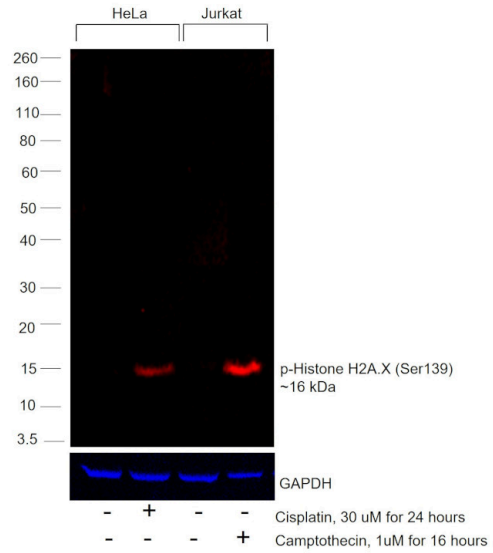
Protocols: We recommend Protocol B: One-step protocol: intracellular (nuclear) proteins. Alternatively, Protocol C: Two-step protocol: Fixation/Methanol can also be used. Protocol A: Two-step protocol: intracellular (cytoplasmic) proteins cannot be used. All Protocols can be found in the "Staining intracellular Antigens for Flow Cytometry Protocol" located in the Best Protocols Section under the Resources tab online.

eFluor® 660 is a replacement for Alexa Fluor® 647. eFluor® 660 emits at 659 nm and is excited with the red laser (633 nm). Please make sure that your instrument is capable of detecting this fluorochome.

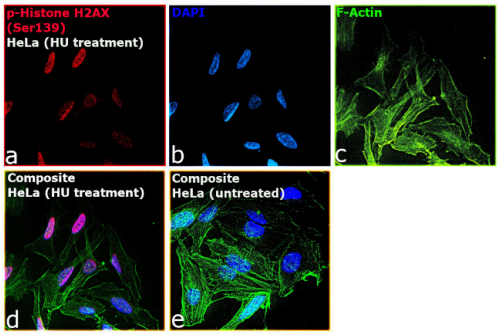
Excitation: 633-647 nm; Emission: 668 nm; Laser: Red Laser.

Filtration: 0.2 µm post-manufacturing filtered.

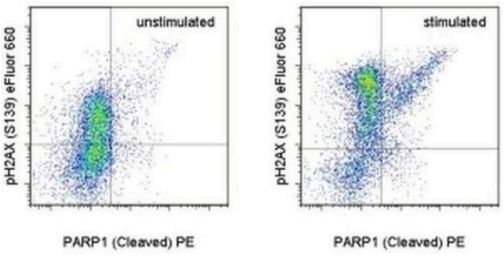
Product Images For Phospho-Histone H2A.X (Ser139) Monoclonal Antibody (CR55T33), eFluor™ 660, eBioscience™



Phospho-Histone H2A.X (Ser139) Antibody (50-9865-42) in WB
Western blot was performed using Phospho-Histone H2A.X (Ser139) Monoclonal Antibody (CR55T33), eFluor 660, eBioscience™ (Product # 50-9865-42) and a 16 kDa band corresponding to phospho-H2A.X was observed on treatment with cisplatin and camptothecin in HeLa and Jurkat cells respectively. Whole cell extracts (30 µg lysate) of HeLa (Lane 1), HeLa treated with cisplatin (30 µM for 24 hr) (Lane 2), Jurkat (Lane 3), Jurkat treated with camptothecin (1 µM for 16 hr) (Lane 4) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX), 10 well. Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001) and blocked using Blocker™ FL Fluorescent Blocking Buffer (10X) (Product # 37565). The blot was probed with the primary antibody (1:400) and fluorescent detection was performed using the iBright™ FL1500 Imaging System (Product # A44115).



Phospho-Histone H2A.X (Ser139) Antibody (50-9865-42) in ICC/IF
Immunofluorescence analysis of phospho-H2A.X was performed using 70% confluent log phase HeLa cells treated with hydroxyurea (3 mM for 6 hr). The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with Phospho-Histone H2A.X (Ser139) Monoclonal Antibody (CR55T33), eFluor 660, eBioscience™ (Product # 50-9865-42, 1:50) in 0.1% BSA, and incubated at 4 degree celsius overnight (Panel a: Red). Nuclei (Panel b: Blue) were stained with ProLong™ Diamond Antifade Mountant with DAPI (Product # P36962). F-actin (Panel c: Green) was stained with Alexa Fluor™ 488 Phalloidin (Product # A12379, 1:300). Panel d represents the merged image showing nuclear localization. Panel e represents untreated HeLa cells. The images were captured at 60X magnification.



Phospho-Histone H2A.X (Ser139) Antibody (50-9865-42) in Flow
Intracellular staining of 3-day unstimulated (left) or 200 uM etoposide-treated (right) normal human peripheral blood cells with Anti-Human PARP1 (Cleaved) PE (Product # 12-6668-42) and Anti-Human/Mouse phospho-H2AX (S139) eFluor® 660 using the Foxp3/Transcription Factor Staining Buffer Set and protocol (Product # 00-5523-00). Cells in the lymphocyte gate were used for analysis.

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6 References

Western Blot (2)

Oncogene

Synergistic lethality between PARP-trapping and alantolactone-induced oxidative DNA damage in homologous recombination-proficient cancer cells.

"Published figure using Phospho-Histone H2A.X (Ser139) monoclonal antibody (Product # 50-9865-42) in Immunohistochemistry"

Authors: Wang H,Zhang S,Song L,Qu M,Zou Z

Year
2020

Molecular oncology

Fibroblast Growth Factor 2 lethally sensitizes cancer cells to stress-targeted therapeutic inhibitors.

"Published figure using Phospho-Histone H2A.X (Ser139) monoclonal antibody (Product # 50-9865-42) in Western Blot"

Authors: Dias MH,Fonseca CS,Zeidler JD,Albuquerque LL,da Silva MS,Cararo-Lopes E,Reis MS,Noël V,Dos Santos EO,Prior IA,Armelin HA

Year
2019

Immunohistochemistry (1)

Oncogene

Synergistic lethality between PARP-trapping and alantolactone-induced oxidative DNA damage in homologous recombination-proficient cancer cells.

"Published figure using Phospho-Histone H2A.X (Ser139) monoclonal antibody (Product # 50-9865-42) in Immunohistochemistry"

Authors: Wang H,Zhang S,Song L,Qu M,Zou Z

Year
2020

Flow Cytometry (3)

Frontiers in oncology

Rational Targeting of Cdc42 Overcomes Drug Resistance of Multiple Myeloma.

"50-9865-42 was used in Flow Cytometry to provide a proof of concept demonstration that rational targeting of Cdc42 represents a promising approach to overcome MM drug resistance."

Authors: Nguyen P,Chakrabarti J,Li Y,Kalim KW,Zhang M,Zhang L,Zheng Y,Guo F

Year
2022

Species
Human

View more Flow references on thermofisher.cn

More applications with references on thermofisher.cn