## **Technical Data Sheet**

# Oligo Mouse Anti-H2AX (pS139)

#### **Product Information**

Material Number: 940519
Size: 25 Tests
Clone: N1-431

Alternative Name: H2A.X; H2A/X; H2AFX; HIST5-2AX; gammα-H2AX; γ-H2AX; H2AX (pS140)

Reactivity: Tested in Development:Human,Mouse

Isotype: Mouse BALB/c IgG1, κ

Application: Single Cell 3' Sequencing Intracellular CITE-seq(Tested During

Development)

Barcode Sequence: AGCCGGTTGAGATGTAGATGTAGATATG

Storage Buffer: Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Regulatory Status: RUO

## **Description**

Histones are highly basic proteins that complex with DNA to form chromatin. The H2AX histone (~15 kDa calculated molecular weight) is a member of the H2A histone family whose members are components of nucleosomal histone octamers. Double-stranded breaks in DNA caused by replication errors, apoptosis, or other physiological processes (including, immunoglobulin and TCR gene recombinations) and DNA damage caused by ionizing radiation, UV light, or cytotoxic agents lead to phosphorylation of H2AX on serine 139. H2AX (pS139) is also referred to as H2AX (pS140) when the N-terminal methionine that is normally excised during posttranslational processing is included in amino acid sequence numbering. Kinases such as ataxia telangiectasia mutated (ATM) or ATM-Rad3-related (ATR) phosphorylate H2AX to induce its function. Phosphorylated H2AX (also termed, gamma-H2AX) functions to recruit and localize DNA repair proteins or cell cycle checkpoint factors to the DNA-damaged sites. In this way, phosphorylated H2AX promotes DNA repair and maintains genomic stability and thus helps prevent oncogenic transformations. Immunofluorescent staining and bioimaging analysis of cultured cells can be used to readily identify H2AX (pS139)-containing foci. As such, H2AX (pS139) immunofluorescence localization serves as a biomarker for nuclear sites of DNA damage (e.g., double-stranded DNA breaks) in affected cells.

#### **Preparation and Storage**

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze. The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography and conjugated to BD® AbSeq oligonucleotide under optimal conditions.

#### **Recommended Assay Procedure**

Put all BD® AbSeq reagents to be pooled into a Latch Rack for  $500 \, \mu L$  Tubes (Thermo Fisher Scientific Cat. No. 4900). Arrange the tubes so that they can be easily uncapped and re-capped with an 8-Channel Screw Cap Tube Capper (Thermo Fisher Scientific Cat. No. 4105MAT) and the reagents aliquoted with a multi-channel pipette. BD® AbSeq tubes should be centrifuged for = 30 seconds at  $400 \times g$  to ensure removal of any content in the cap/tube threads prior to the first opening.

When using BD® AbSeq intracellular markers with the Single Cell 3' Sequencing Intracellular CITE-seq, cells must first be fixed and permeabilized using the BD Rhapsody™ Intracellular AbSeq Buffer Kit before the antibody-oligo can bind to the protein. Refer to the list of required companion products below and see BD Rhapsody™ System Single-Cell Labelling with BD® AbSeq Ab-Oligos for Intracellular CITE-seq (Doc ID: 23-24464) for the complete BD® AbSeq intracellular multiomics staining protocol. Contact your local Field Application Specialist (FAS) for additional guidance.

Use standard laboratory safety protocols. Read and understand the safety data sheets (SDSs) before handling chemicals. To obtain SDSs, go to regdocs.bd.com or contact BD Biosciences technical support at scomix@bdscomix.bd.com. Warning: All biological specimens and materials contacting them are considered biohazardous. Handle as if capable of transmitting infection and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

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## **Suggested Companion Products**

Catalog Number	Name	Size
570742	Intracellular AbSeq Buffer Kit	1 Each
570750	AbSeq Enhancer Kit	1 Each
570908	OMICS-Guard Sample Preservation Buffer Kit	12 Each
570751	RNase Inhibitor	1 Each
633801	Whole Transcriptome Analysis (WTA) Amplification Kit	1 Each
554656	Stain Buffer (FBS)	500 mL
666262	8-Lane Cartridge	1 Each
564219	Human BD Fc Block™	50 μg
664887	Enhanced Cartridge Reagent Kit	1 Each
633733	Cartridge Kit	1 Each
633773	cDNA Kit	1 Each
633781	Hu Single Cell Sample Multiplexing Kit	1 Each
570911	OMICS-Guard Sample Preservation Buffer	50 mL
633707	Express Single-Cell Analysis System Package	1 EA
633701	Single-Cell Analysis System	1 EA

#### **Product Notices**

- 1. Please refer to www.bdbiosciences.com/us/s/resources for technical protocols.
- 2. This reagent has been pre-diluted for use at the recommended volume per test. Typical use is 2  $\mu$ l for 1 × 10<sup>6</sup> cells in a 200- $\mu$ l staining reaction.
- 3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
- 4. The production process underwent stringent testing and validation to assure that it generates a high-quality conjugate with consistent performance and specific binding activity. However, verification testing has not been performed on all conjugate lots.
- 5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
- 6. Species cross-reactivity detected in product development may not have been confirmed on every format and/or application.
- 7. Please refer to http://regdocs.bd.com to access safety data sheets (SDS).
- 8. Please refer to bd.com/genomics-resources for technical protocols.
- 9. Illumina is a trademark of Illumina, Inc.
- 10. For U.S. patents that may apply, see bd.com/patents.

#### References

Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ. ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem. 2001; 276(45):42462-42467. (Biology).

Fernandez-Capetillo O, Lee A, Nussenzweig M, Nussenzweig A. H2AX: the histone guardian of the genome. DNA Repair (Amst). 2004; 3(8-9):959-967. (Biology).

Kuo LJ, Yang LX. Gamma-H2AX - A novel biomarker for DNA double-strand breaks. In Vivo. 2008; 22(3):305-309. (Biology). Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. J Biol Chem. 2000; 275(13):9390-9395. (Biology).

Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem. 1998; 273(10):5858-5868. (Biology).

Austin WR, Armijo AL, Campbell DO, et al.. Nucleoside salvage pathway kinases regulate hematopoiesis by linking nucleotide metabolism with replication stress. J Exp Med. 2012; 209(12):2215-2228. (Clone-specific: Flow cytometry). Lavelle D, Vaitkus K, Ling Y, et al. Effects of tetrahydrouridine on pharmacokinetics and pharmacodynamics of oral decitabine. Blood. 2012; 119(5):1240-1247. (Clone-specific: Flow cytometry).

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