BD Pharmingen™

Bioimaging Certified Reagent

Technical Data Sheet

Alexa Fluor® 647 Rat anti-Histone H3 (pS28)

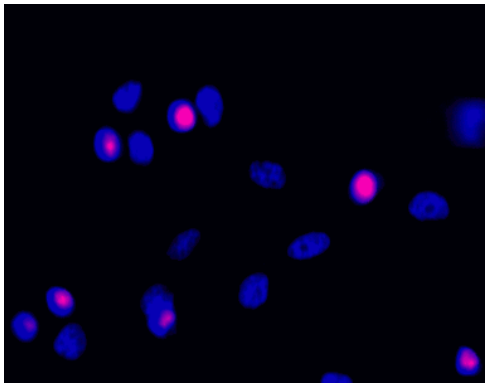
Product Information

Material Number:	**558609**
Size:	100 tests
Vol. per Test:	5 µl
Clone:	HTA28
Immunogen:	Phosphorylated Human Histone H3 Peptide
Isotype:	Rat IgG2a, κ
Reactivity:	Tested: Human Reported: Cow, Drosophila, Mouse, Rat
Storage Buffer:	Aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Description

Histones are highly basic proteins that complex with DNA to form chromatin. Histone H3 is a ~15-kDa protein that is phosphorylated at serine 28 (S28), S10, and/or threonine 11 during mammalian cell mitosis and meiosis. The phosphorylation sites are located in the N-terminal tail, a region that is outside of the chromatin fiber and is thus accessible for interactions with agents that may regulate chromatin or specific gene activities. The phosphorylation states of the two serine sites during the cell cycle are highly regulated by Aurora B kinase and a PP1 phosphatase: S10 is in the phosphorylated state from late G2 phase to anaphase, while S28 is phosphorylated from prophase to anaphase. Furthermore, phosphorylation of histone H3 S28 may be mediated by other kinases in response to external stimuli. Evidence suggests that histone phosphorylation is involved in the regulation of chromosome condensation, cell division, and gene transcription.

The HTA28 monoclonal antibody reacts with histone H3 phosphorylated at S28 in its N-terminal tail. It does not recognize the unphosphorylated protein.



Immunofluorescent staining of human cell lines. *HeLa cells (ATCC CCL-2) were cultured overnight and treated for 4 hours with 15 ng/ml colchicine to increase the proportion of mitotic cells. Then they were fixed, permeabilized with cold methanol, stained with Alexa Fluor® 647 Rat anti-Histone H3 (pS28) (pseudo-colored red, which appears pink when co-localized with the blue) and counter-stained with Hoechst 33342 (pseudo-colored blue) according to the Recommended Assay Procedure. The images were captured on a BD Pathway™ 855 Bioimager System with a 20x objective and merged using BD Attovision™ software. This antibody also stains A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells, and it works with either cold methanol or Triton X-100 permeabilization (see Recommended Assay Procedure).*

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.

The antibody was conjugated to Alexa Fluor® 647 under optimum conditions, and unreacted Alexa Fluor® 647 was removed.

Application Notes

Application

Bioimaging	Routinely Tested

Recommended Assay Procedure:

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) to each well and incubating for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either cold methanol or Triton™ X-100:
    a. Add 100 µl of -20°C 90% methanol or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

    OR

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b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
Triton is a trademark of The Dow Chemical Company.

4. Remove the permeabilizer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of blocking buffer (3% FBS in 1× PBS) or BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well and incubating for 30 minutes at RT.
6. Remove the blocking buffer, dilute the antibody conjugate 1:10 in blocking buffer or Stain Buffer (FBS), and stain the cells by adding 50 µl of the diluted antibody conjugate to each well and incubating for 1 hour at RT.
7. Remove the diluted antibody conjugate, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and counter-stain the nuclei by adding 100 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
9. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
353219	BD Falcon™ 96-well Imaging Plate	1 box	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. This reagent has been pre-diluted for use at the recommended Volume per Test. We typically use 1 × 10^6 cells in a 100-µl experimental sample (a test).
2. This product is sold under license from Shigei Medical Research Institute, Okayama, Japan.
3. Alexa Fluor is a registered trademark of Molecular Probes, Inc., Eugene, OR.
4. The Alexa Fluor®, Pacific Blue™, and Cascade Blue® dye antibody conjugates in this product are sold under license from Molecular Probes, Inc. for research use only, excluding use in combination with microarrays, or as analyte specific reagents. The Alexa Fluor® dyes (except for Alexa Fluor® 430), Pacific Blue™ dye, and Cascade Blue® dye are covered by pending and issued patents.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. 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.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

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