Technical Data Sheet

V450 Rat Anti-Histone H3 (pS28)

Product Information

Material Number:	**560606**
Size:	50 tests
Vol. per Test:	5 µl
Clone:	HTA28
Immunogen:	Phosphorylated Human Histone H3 Peptide
Isotype:	Rat IgG2a, κ
Reactivity:	QC Testing: Mouse Predicted: Human, Rat, Hamster, Cow
Storage Buffer:	Aqueous buffered solution containing protein stabilizer and ≤0.09% sodium azide.

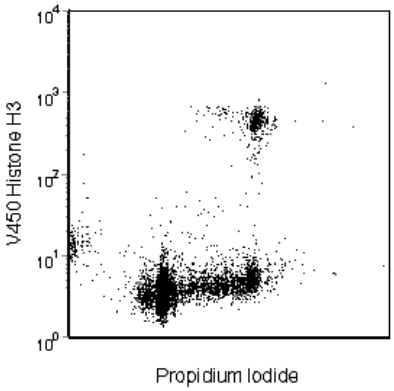
Description

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.

HTA28 has been predicted to be reactive on human, rat, hamster, and cow samples. Investigators are advised that the BD Hoirzon™ V450 Rat Anti-Histone H3 (pS28) antibody (clone HTA28) is not routinely tested on human, rat, hamster, and cow samples.

The antibody is conjugated to BD Horizon™ V450, which has been developed for use in multicolor flow cytometry experiments and is available exclusively from BD Biosciences. It is excited by the **V**iolet laser Ex max of 406 nm and has an Em Max at **450** nm. Conjugates with BD Horizon™ V450 can be used in place of Pacific Blue™ conjugates.



Flow cytometric analysis of Histone H3 (pS28) on C20 cells. *C20 cells, a mouse T-helper lymphocytic cell line with cytotoxic activity, were treated with 1 µg/mL Colcemid (Sigma-Aldrich Cat. No. D7385; also known as Demecolcine or N-Deacetyl-N-methylcolchicine) for 4 hours at 37 °C to increase the population of mitotic cells. Cells were then fixed and permeabilized with 70% cold ethanol prior to staining with the Rat Anti-Histone H3 (pS28) antibody in conjunction with RNase/propidium iodide. Dot plots were derived from gated events based on light scattering characteristics for C20 cells. Flow cytometry was performed on a BD™ LSR II flow cytometry system.*

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 with BD Horizon™ V450 under optimum conditions, and unreacted BD Horizon™ V450 was removed.

BD Biosciences

bdbiosciences.com

United States	Canada	Europe	Japan	Asia Pacific	Latin America/Caribbean
877.232.8995	888.268.5430	32.53.720.550	0120.8555.90	65.6861.0633	0800.771.7157

For country-specific contact information, visit **bdbiosciences.com/how_to_order/**

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Flow cytometry: Colcemid (Demecolcine) influences cells by depolymerizing microtubules and arresting the cell cycle (i.e spindles cannot form) at the metaphase checkpoint during mitosis, also known as the M-phase. Investigators may want to consider using Colcemid for purposes of increasing the frequency of cells found in the M-phase as identifying these cells while they cycle normally can be difficult due to low population frequencies. As Histone H3 is phosphorylated during the M-phase, the BD Horizon™ V450 Rat Anti-Histone H3 (pS28) antibody may be useful for helping identify these cells that are in the M-phase.

1. Wash cell suspension twice with 1X PBS.
2. Fix the cells by adding ice-cold 70% ethanol drop-wise while vortexing the cell suspension, then storing them for at least 4 hours at -20°C in the 70% ethanol.
3. Aliquot ~1 million fixed cells per tube for staining. Wash them twice with 1X PBS, then once with stain buffer.
4. Stain the cells with 5 µl BD Horizon™ V450 Rat Anti-Histone H3 (pS28) in 95 µl stain buffer for 20 minutes at room temperature, then wash them with stain buffer.
5. For optimum cell cycle analysis, the cells should be treated with RNase before staining with propidium iodide. Investigators may wish to stain the cells with 0.5 ml PI/RNase Staining Buffer (Cat. No. 550825) for 15 minutes at room temperature or alternatively, treat the stained cells with 50 µg RNase A (Sigma-Aldrich Cat. No. R5500) in 50 µl 1X PBS for 30 minutes at 37°C and without washing, stain DNA by adding 5 µg Propidium Iodide (Sigma-Aldrich Cat. No. P4170) in 450 µl staining buffer for at least 10 minutes at room temperature.
6. The cells are now ready for flow cytometric analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
550825	PI/RNase Staining Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 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. BD Horizon™ V450 has a maximum absorption of 406 nm and maximum emission of 450 nm. Before staining with this reagent, please confirm that your flow cytometer is capable of exciting the fluorochrome and discriminating the resulting fluorescence.
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. Pacific Blue™ is a trademark of Molecular Probes, Inc., Eugene, OR.
5. This product is sold under license from Shigei Medical Research Institute, Okayama, Japan.
6. For fluorochrome spectra and suitable instrument settings, please refer to our Fluorochrome Web Page at www.bdbiosciences.com/colors.
7. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Carmena M, Earnshaw WC. The cellular geography of aurora kinases. *Nat Rev Mol Cell Biol.* 2003; 4:842-854. (Biology)

Cheung P, Allis CD, Sassone-Corsi P. Signaling to chromatin through histone modifications. *Cell.* 2000; 103:263-271. (Biology)

Choi HS, Choi BY, Cho Y-Y, Zhu F, Bode AM, Dong Z. Phosphorylation of Ser28 in histone H3 mediated by mixed lineage kinase-like mitogen-activated protein triple kinase α. *J Biol Chem.* 2005; 280(14):13545-13553. (Biology)

Dyson MH, Thomson S, Inagaki M, et al. MAP kinase-mediated phosphorylation of distinct pools of histone H3 at S10 or S28 via mitogen- and stress-activated kinase 1/2. *J Cell Sci.* 2005; 118(10):2247-2259. (Biology)

Furukawa K, Sugiyama S, Osouda S, et al. Barrier-to-autointegration factor plays crucial roles in cell cycle progression and nuclear organization in Drosophila. *J Cell Sci.* 2003; 116(18):3811-3823. (Biology)

Goto H, Tomono Y, Ajiro K, et al. Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. *J Biol Chem.* 1999; 274(36):25543-25549. (Immunogen)

Hirata A, Inada K, Tsukamoto T, et al. Characterization of a monoclonal antibody, HTA28, recognizing a histone H3 phosphorylation site as a useful marker of M-phase cells. *J Histochem Cytochem.* 2004; 52(11):1503-1509. (Biology)

Nowak SJ, Corces VG. Phosphorylation of histone H3: a balancing act between chromosome condensation and transcriptional activation. *Trends Genet.* 2004; 20(4):214-220. (Biology)

Pascreau G, Arlot-Bonnemains Y, Prigent C. Phosphorylation of histone and histone-like proteins by aurora kinases during mitosis. *Prog Cell Cycle Res.* 2003; 5:369-374. (Biology)