Technical Data Sheet

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

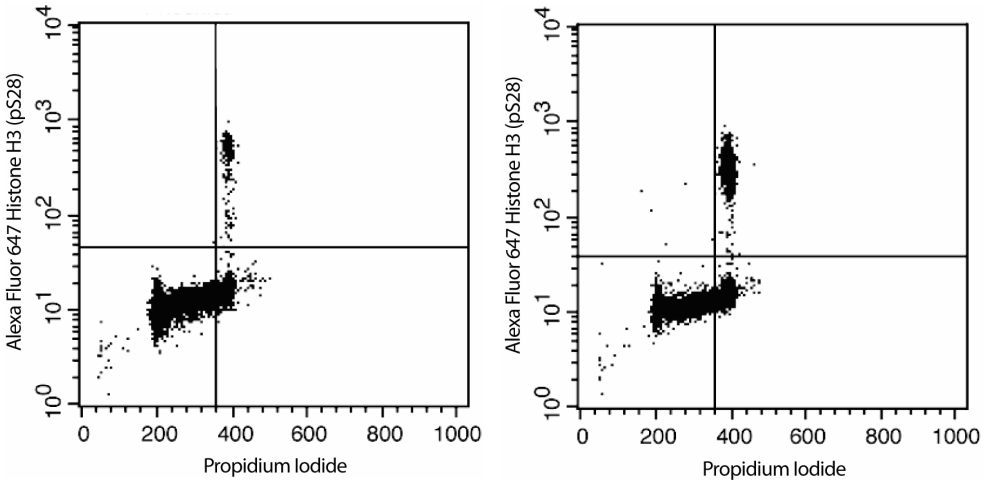
Product Information

Material Number:	**558217**
Size:	50 tests
Vol. per Test:	20 µl
Clone:	HTA28
Immunogen:	Phosphorylated Human Histone H3 Peptide
Isotype:	Rat IgG2a, κ
Reactivity:	QC Tested: Mouse Tested in Development: Human Reported: Cow, Drosophila, Hamster, 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.



Analysis of Histone H3 (pS28) in proliferating mouse T lymphocytes. *A log phase mouse cytotoxic T cell line was either untreated (left panel) or cultured with 1 µg/ml of the microtubule depolymerizer demecolcine (Sigma D7385) for 4 hrs (right panel). The cells were fixed and stained according to the Recommended Assay Procedure. Flow cytometry was performed on a BD FACSCalibur™ flow cytometry system. In this example, the demecolcine treatment increased the frequency of M phase cells from 20% to 52%, and the frequency of M phase cells expressing Histone H3 (pS28) increased from 15% to 51% (upper right quadrants of the left and right panels, respectively).*

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

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

Recommended Assay Procedure

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.

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4. Stain the cells with 20 µl Alexa Fluor® 647 Rat anti-Histone H3 (pS28) in 80 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:
   - Treat the stained cells with 50 µg RNAse A (Sigma R5500) in 50 µl 1X PBS for 30 minutes at 37°C. Without washing, stain DNA by adding 5 µg Propidium Iodide (Sigma P4170) in 450 µl staining buffer for at least 10 minutes at room temperature.

   Or
   - Stain the cells with 0.5 ml PI/RNase Staining Buffer (Cat. No. 550825) for 15 minutes at room temperature.
6. The cells are now ready for flow cytometric analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554656	Stain Buffer (FBS)	500 ml	(none)
550825	PI/RNase Staining Buffer	100 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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This product is sold under license from Shigei Medical Research Institute, Okayama, Japan.
4. Alexa Fluor® 647 fluorochrome emission is collected at the same instrument settings as for allophycocyanin (APC).
5. For fluorochrome spectra and suitable instrument settings, please refer to our Multicolor Flow Cytometry web page at www.bdbiosciences.com/colors.
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. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
8. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR.
9. 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.

References

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