

MONOCLONAL ANTIBODY

Anti-Histone H2B mAb

Code No.	Clone	Subclass	Quantity	Concentration
D212-3	C14264	Mouse IgG2b	100 µL	1 mg/mL

BACKGROUND: The nucleosome is made up of four core histone proteins (H2A, H2B, H3 and H4) and is the primary building block of chromatin. The N-terminal tail of core histones undergoes multiple different post-translational modifications including acetylation, phosphorylation, ubiquitination, methylation, and ADP-ribosylation. These modifications occur in response to cell signal stimuli and have a direct effect on gene expression. Acetylation of histone H2B was suppressed to greater extent than that of the others, with histone H3 being relatively least affected. The analysis of acetylation status of each of the four lysine sites at the N-terminal tail of histone H2B revealed decreases consistent with those observed in the total acetylation patterns, with the K12 and K20 residues being markedly more affected than K5 and K15 residues.

SOURCE: This antibody was purified from hybridoma (clone C14264) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag14 with Balb/c mouse splenocyte immunized with the protein fraction extracted from the mitotic chromosome.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with Histone H2B (14 kDa) on Western blotting, Immunoprecipitation, and Immunocytochemistry.

APPLICATIONS:

Western blotting: 1 µg/mL for chemiluminescence detection system

Immunoprecipitation: 2 µg/200 µL of cell extract from 5x10⁶ cells

Immunohistochemistry: Not tested

Immunocytochemistry: 1 µg/mL

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOLS.**

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

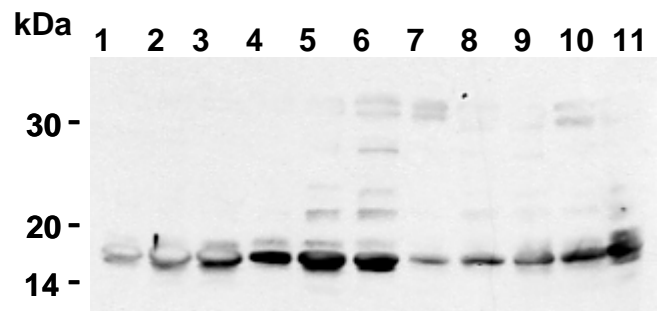
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Hamster
Cells	Jurkat, HeLa, Raji, HL-60, A431	L5178Y, WR19L, NIH/3T3	PC12, Rat1	CHO
Reactivity on WB	+	+	+	+

REFERENCES:

- 1) Higashi, T., *et al.*, *J. Biotechnol.* **120**, 262-272 (2005)
- 2) Maresca, T. J., *et al.*, *J. Cell Biol.* **169**, 859-869 (2005)
- 3) Uchiyama, S., *et al.*, *J. Biol. Chem.* **280**, 16994-17004 (2005)
- 4) Vettese-Dadey, M., *et al.*, *EMBO J.* **15**, 2508-2518 (1996)
- 5) van Holde, K. E., *Chromatin (Springer-Verlag, Berlin)* 69-343 (1988)

Clone C14264 is used in reference number 1).



Western blot analysis of Histone H2B expression in Jurkat (1), HeLa (2), Raji (3), HL-60 (4), A431 (5), L5178Y (6), WR19L (7), NIH/3T3 (8), CHO (9), PC12 (10) and Rat1 (11) using D212-3.

PROTOCOLS:

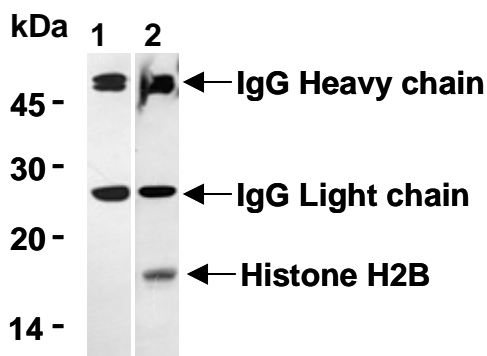
SDS-PAGE & Western Blotting

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C

and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.

- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 3 minutes and centrifuge. Load 10 μL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm² for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
- 9) Incubate the membrane with 1:10,000 of Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (10 minutes x 3 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
- 12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 13) Expose to an X-ray film in a dark room for 3 minutes.
- 14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, HeLa, Raji, HL-60, A431, NIH/3T3, L5178Y, WR19L, PC12, Rat1 and CHO)

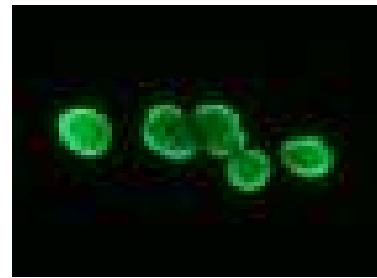


Immunoprecipitation of Histone H2B from HeLa cells with mouse IgG2b (1) or D212-3 (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D212-3.

Immunoprecipitation

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
- 3) Add primary antibody as suggest in the **APPLICATIONS** into 200 μL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C.
- 4) Add 20 μL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
- 5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
- 6) Resuspend the beads in 20 μL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 10 μL /lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; HeLa)



Immunocytochemical detection of Histone H2B on 4% PFA fixed HeLa cells with D212-3.

Immunofluorescence microscopy

- 1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1×10^4 cells for one slide, then incubate in a CO₂ incubator for one night.)
- 2) Wash the cells 3 times with PBS.
- 3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 20 minutes at room temperature.
- 4) Wash the glass slide 3 times with PBS.
- 5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
- 6) Wash the glass slide 3 times with PBS.
- 7) Add the primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
- 8) Wash the glass slide 3 times with PBS.
- 9) Add 100 μL of 1:100 Anti-IgG (Mouse) pAb-FITC (MBL; code no. 238) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out

light by aluminum foil.

- 10) Wash the glass slide 3 times with PBS.
- 11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
- 12) Promptly add Permafluor™ aqueous mounting medium (MBL; code no. IM-0752) onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; HeLa)

RELATED PRODUCTS:

- D209-3 Anti-Histone H1 mAb (C14093)
- D210-3 Anti-Histone H2A mAb (C10037)
- D212-3 Anti-Histone H2B mAb (C14264)
- PM006 Anti-Phospho-Histone H3 (Ser28) (Human) pAb (Polyclonal)
- D214-3 Anti-Histone H4 mAb (C14691)
- D215-3 Anti-NuMA mAb (C02142)
- D216-3 Anti-hn-RNP-A2/B1 mAb (C20308)
- D075-3 Anti-HMGB1 (HMG1) mAb (KS1)
- D090-3 Anti-HMGB1/2 (HMG1/2) mAb (FBH7)
- K0115-3 Anti-CAF-1 p150 (Human) mAb (p150-48)
- K0116-3 Anti-CAF-1 p60 (Human) mAb (p60-24)
- CY-P1011 Anti-Histone Deacetylase 1 pAb (Polyclonal)
- CY-P1015 Anti-Phospho-Histone-H2A.X (Ser139) pAb (Polyclonal)
- CY-M1029 Anti-Acetylated Histone/p53 (Lys382) mAb (TM-5C5)
- CY-1140 CycLex® Cellular Histone Acetylation Assay Kit
- CY-1143 CycLex® Histone H2A.X Phosphorylation Cellular ELISA Kit