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Product datasheet for AM26525AF-N

HIST4H4 Mouse Monoclonal Antibody [Clone ID: C14691]

Product data:

Product Type:	Primary Antibodies
Clone Name:	C14691
Applications:	IF, IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 5 µg/200 µl of cell extract from 5x10^6 cells. **Immunocytochemistry:** 5 µg/ml.
Reactivity:	Hamster, Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG2b
Clonality:	Monoclonal
Immunogen:	Protein fraction extracted from the mitotic chromosome.
Specificity:	This antibody reacts with Histone H4.
Formulation:	PBS containing 50% Glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid purified Ig fraction
Concentration:	lot specific
Purification:	Protein A Agarose Chromatography
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Predicted Protein Size:	11 kDa
Gene Name:	histone cluster 4, H4
Database Link:	Entrez Gene 121504 Human P62805

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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-transcriptional 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 H4 plays a primary role in the structural changes that mediate enhanced binding of transcription factors to their recognition sites within nucleosomes. It has been suggested that acetylated Histone H4 also is involved in heightened activation of the transcription of male X chromosomes, and that Histone H4 stimulates glucose transport activity in rat skeletal muscle.

Synonyms: HIST1H4, H4/A, H4FA

Note: This product was originally produced by MBL International.

Protocol:

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 4oC 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 4oC 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 manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 5% 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 10 % Block Ace 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 the 1:10,000 HRP-conjugated anti-mouse IgG diluted with 10% Block Ace 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; HeLa, Raji, HL-60, A431, NIH/3T3, L5178Y, WR19L, PC12, Rat1, CHO).

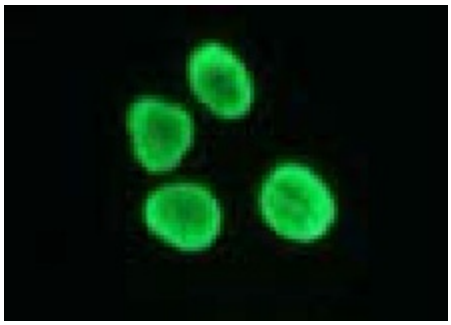
Immunoprecipitation

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 4oC 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 4oC 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).

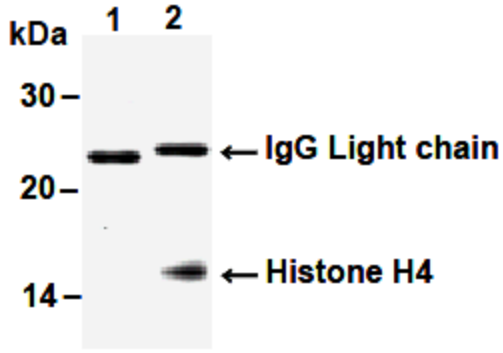
Immunofluorescence microscopy

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread $1x10^4$ cells for one slide, then incubate in a CO_2 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) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) The glass slide was washed 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 are recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add 100 µL of 1:100 FITC conjugated anti-mouse IgG diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 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 PermafluorTM aqueous mounting medium onto the slide, then put a cover slip on it.
(Positive control for Immunocytochemistry; HeLa).

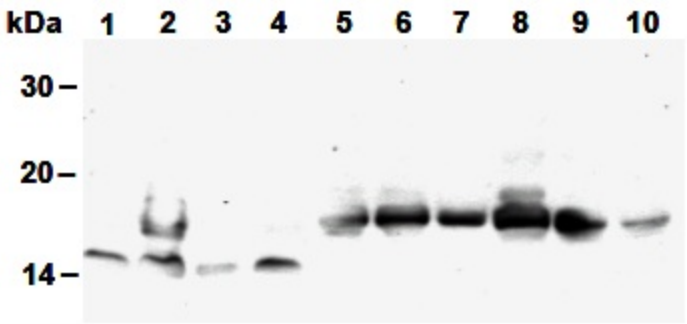
Product images:



Immunocytochemical detection of Histone H4 on 4% PFA fixed HeLa cells with AM26525AF-N.



Immunoprecipitation of Histone H4 from HeLa cells with Mouse IgG2b (1) or AM26525AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26525AF-N.



Western blot analysis of Histone H4 expression in HeLa (1), Raji (2), HL-60 (3), A431 (4), L5178Y (5), WR19L (6), NIH/3T3 (7), CHO (8), PC12 (9) and Rat1 (10) using AM26525AF-N.