OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26685AF-N

Histone H2A Mouse Monoclonal Antibody [Clone ID: C10037]

Product data:

Product Type:	Primary Antibodies
Clone Name:	C10037
Applications:	IF, IP, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunoprecipitation:** 2 µg/200 µl of cell extract from 5x10^6 cells. **Immunocytochemistry:** 1 µg/ml. For details ***See Protocols*** below.
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 H2A.
Formulation:	PBS, pH 7.2 containing 50% Glycerol State: Azide Free State: Liquid puirfied Ig fraction Preservative: None
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Predicted Protein Size:	14 kDa

View online »

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 multIPPle 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. The role of histone variants, and specially those of H3 and H2A, in various nuclear processes has been long appreciated. There are at least three different families of H2A variants present in a variety of organisms from yeast to mammals, and the degree of conservation among members of each family is greater than that of the canonical H2A. H2AX is thought to play a role in DNA double-strand break repair; the serine in the SQEY motif of H2AX is phosphorylated at the site of the DNA damage and serves as a signal for the recruitment of repair proteins. Macro H2A1, another H2A variant, has been shown to have a role in X-chromosome inactivation and dosage compensation in mammals, where it is found to localize to the inactive X after silencing has been established.

Synonyms: H2A Histone Family, HIST1H2A

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 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 manufacture'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 the 1:10,000 HRP-conjugated anti-mouse IgG 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; HeLa, Raji, HL-60, A431, L5178Y, WR19L, NIH/3T3, PC12, Rat1, CHO)

Immunoprecipitation

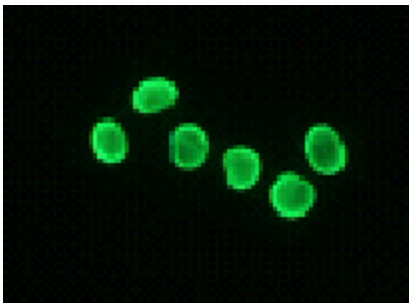
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.05% NP-40) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 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 300 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4oC. 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.
4) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
5) 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

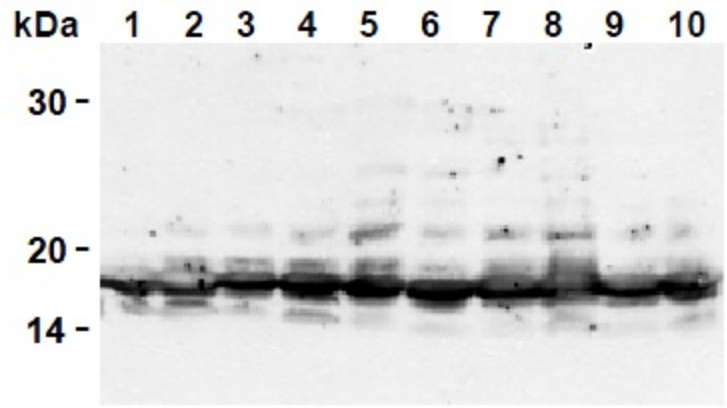
1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10e4 cells for one slide, then incubate in a CO2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde (PFA) for 20 minutes at room temperature.
4) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% Triton X-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)

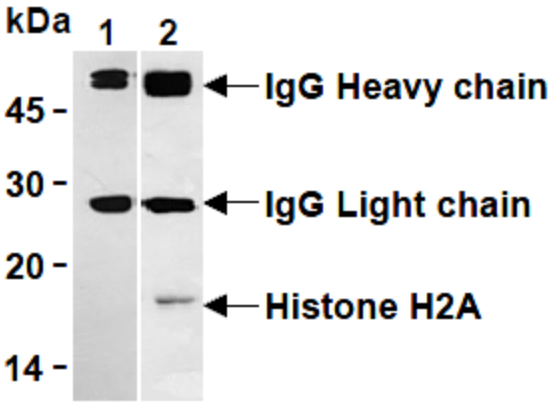
Product images:



Immunocytochemical detection of Histone H2A on 4% PFA fixed HeLa cells with AM26685AF-N.



Western blot analysis of Histone H2A 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 AM26685AF-N.



Immunoprecipitation of Histone H2A from HeLa cells with mouse IgG2b (1) or AM26685AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with AM26685AF-N.