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Product datasheet for AM26583AF-N

MSX2 (1-77) Mouse Monoclonal Antibody [Clone ID: 2E12]

Product data:

Product Type:	Primary Antibodies
Clone Name:	2E12
Applications:	IHC, WB
Recommended Dilution:	**Western blot:** 1:1000 for chemiluminescence detection system (The myc-tagged-Msx2 overexpressed cell lysate was derived from pCMV-myc-tagged-Msx2 transfected 293T cells). **Immunohistochemistry on frozen sections:** 1-10 µg/ml (Antibody was frozen sections of new born rat cranium).
Reactivity:	Human, Rat
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Recombinant protein corresponding to N-terminal amino acids (1-77 a.a.) of human Msx2
Specificity:	Anti-Msx2 detects the 9 kDa Myc-tagged-human Msx2 (N-terminal) expressed in 293T cell by Western blot.
Formulation:	PBS containing 50% glycerol, pH 7.2. Contains no preservatives. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	msh homeobox 2
Database Link:	Entrez Gene 4488 Human P35548

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Background: Homeobox genes (HOX gene), which encode DNA binding proteins that recognize specific sequences and modulate transcriptional activity, are expressed during embryogenesis with positional specificity 1). HOX-8 (Msx2) mRNA is highl y expressed in immature tumors, including a yolk sac tumor, seminoma and choriocarcinoma, and that its expression is higher in various tumors of epithelial origin than in the corresponding normal tissues 2). Msx2 gene expression is frequently activated in carcinoma-derived cell lines. The gene is inactive in NIH3T3 cells but is transcriptionally activated after transformation by v-Ki-ras, suggesting that the Msx2 may play a positive role in cell transformation 3).

Synonyms: MSX-2, HOX8, HOX-8

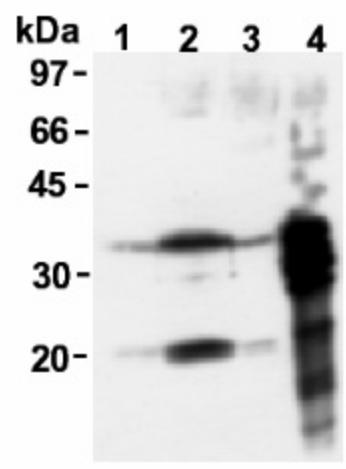
Note: This product was originally produced by MBL International.

Protocol:
SDS PAGE & Western Blotting
1) Boil all samples for 3~5 minutes. Load 10 µl of cell lysate or tissue homogenate (5~20 µg total protein) to each well of an SDS-polyacrylamide gel and electrophorese in a 1 mm thick gel.
2) Transfer to a polyvinylidene difluoride (PVDF) membrane at 10V for 1hour in a semi-dry transfer system. (Transfer Buffer: 25mM Tris, 190mM glycine, 20% MeOH).
3) The transferred proteins can be visualized by staining the membrane for 1 minute with Ponceau S. Rinse the membrane with PBS.
4) Non-specific binding sites are blocked by immersing the membrane in 5% Skim Milk / PBS / 0.05% Tween20 for 1 hour at room temperature, or for overnight at 4°C.
5) Incubate in primary antibody diluted as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal antibody concentration will depend on the experimental conditions and the abundance of the antigen.)
6) Wash the membrane 3 times with PBS, 0.05% Tween20 for 5~10 minutes per wash.
7) Incubate in Horseradish Peroxidase conjugated goat anti-mouse diluted 1:3000 in PBS, 0.05% Tween20 for 45 minutes at room temperature.
8) Wash the membrane 3 times with PBS, 0.05% Tween20 for 10 minutes per wash.
9) Incubate in Amersham ECL Reagent for 1 minute. Drain membrane, remove excess ECL Reagent by dabbing with a Kimwipe, and seal in plastic wrap.
10) Expose to ECL hyperfilm in a dark room for 30 seconds. Develop as usual for autoradiogram or X-ray. The conditions for development and exposure may vary.
IMMUNOHISTOCHEMICAL STAINING For tissue section:
1) Deparaffinize section, hydrate to water.
2) Wash in PBS for 5 minutes.
3) Remove slides from PBS and cover each section with 100~200 µl of H 2 O 2 for 5 minutes to block endogenous peroxidase activity. Do not treat frozen section with 3% H 2 O 2 . Wash in PBS for 2 minutes.
4) Remove slides from PBS, wipe gently around each section and cover tissues with 100~200 µ l of blocking buffer (10% Goat serum in PBS) for 5 minutes to block non specific antibody

staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with 100~200 µl of primary antibody at the concentration suggested in APPLICATIONS in blocking buffer. (The optimal antibody concentration will depend on the experimental conditions and the abundance of the antigen.)
6) Incubate the section for 1 hour at room temperature (37°C for 10 minutes).
7) Wash the slide by gentle removing antibody with a stream from a wash bottle or pipet containing buffer: do not hit tissue section. Wash 3 times with PBS for 1~2 minutes each.
8) Wipe gently around each section and cover tissues with 100~200 µl of Biotinyl-Goat anti-mouse IgG (H+L)(Fab') 2 diluted 1:200 in blocking buffer.
9) Incubate for 30 minutes at room temperature.
10) Wash as in 7.
11) Wipe gently around section and cover tissues with 100~200 µ
l of Streptavidin conjugated HRP diluted 1:2000~4000 in blocking buffer.
12) Incubate for 30 minutes at room temperature.
13) Wash as in 7. 14) Visualize with DAB substrate / chromogen (25mg of DAB in 100ml of PBS plus 2~3ml of 0.3% H 2 O 2) solution for approximately 1~8 minutes. Wash in distilled water.
*DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
15) Counterstain in hematoxylin or methylgreen.
16) Mounting For cultured cell: Immunofluorescence staining Fixing: 1) or 2)
1) Formaldehyde: Rinse the cells on glass coverslips in phosphate buffered saline(PBS), and immerse for 10 minutes in 4% formaldehyde diluted in PBS / 0.2% Triton X-100. Then rinse the coverslips three times for 5 minutes each in PBS.
2) Methanol /acetone: Fix and permeabilize the cells by immersing the cover slip in -20°C methanol/acetone (1:1) for 10 minutes. Air dry.
Blocking:
1) Cover the cells with 0.2% BSA in PBS for 10 minutes to minimize non-specific adsorption of the antibodies to the cover slip (25~50µl is usually sufficient).
Staining:
1) Remove the blocking buffer.
2) Incubate in primary antibody at the concentration suggested in APPLICATIONS, diluted in blocking buffer for 1 hour at room temperature. (The concentration of antibody to be used will depend on several variables and the abundance of the antigen.)
3) Wash the cell 3 times in PBS for 10 minutes each.
4) Incubate in FITC-conjugated anti-mouse IgG diluted 1:160 in blocking buffer for 45 minutes at room temperature.
5) Wash the cell 3 times in PBS for 10 minutes each.
6) Mounting.
For cultured cell: Immunoperoxidase staining Fixing, blocking and staining
1)~3): Same as immunofluorescence staining.
4) Incubate in Horseradish Peroxidase conjugated goat anti-mouse diluted in 1:1000 in blocking buffer for 45 minutes at room temperature.
5) Wash the cell 3 times in PBS for 10 minutes each.
6) Visualize with DAB substrate / chromogen (25mg of DAB in 100ml of PBS plus 2~3ml of H 2

O 2) or AEC solution for approximately 1~8 minutes. Wash in distilled water.
*DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
*7) Counterstain in hematoxylin or methylgreen.
*8) Mounting.

Product images:



Western blot analysis of Msx2 expression in HeLa (1), ZR-75-1 (2), KB (3) and recombinant (4) using AM26583AF-N