

Anti-DNA, single stranded specific, clone F7-26

Monoclonal Antibody

Cat. # MAB3299

Lot # LV1505484

pack size: 50 µg

Store at -80°C

FOR RESEARCH USE ONLY



Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
IH, IH(P), FC, ELISA	A	IgM	N/A	M	N/A	N/A

Background

Apoptosis is a genetically and biochemically regulated mechanism of programmed cell death that plays an important role in a variety of human diseases including cancer, immune disorders, and neurologic, cardiovascular and infectious diseases. Decreased stability of apoptotic DNA toward thermal denaturation is induced by the proteolysis of DNA-bound proteins during the execution of apoptosis. This conclusion is supported by the binding of anti-ssDNA monoclonal antibodies to non-apoptotic cells treated with proteinase K before heating and by prevention of DNA denaturation and MAb reactivity in apoptotic nuclei reconstituted with histones (Frankfurt et al. 1996, 1997). Thus, staining of cell suspensions and tissue sections with such antibodies following a heat treatment, which induces DNA denaturation *in situ* only in apoptotic nuclei, is a specific and sensitive method for the detection of apoptotic cells. CHEMICON MAB3299 provides a cellular marker specific for apoptotic death that is independent of internucleosomal DNA fragmentation and is useful for the detection of different stages of apoptosis in various cell types.

Presentation

Purified mouse monoclonal IgM in buffer containing PBS supplemented with 5% fetal bovine serum (FBS), containing no preservatives.

Concentration

100 µg/mL

Specificity

Antibody MAB3299 is specifically reactive with single-stranded DNA and is ideal for the detection of apoptotic cells in suspension or in tissue sections. The antibody does not recognize DNA in double-stranded conformations. MAB3299 provides a cellular marker specific for apoptotic death that is independent of internucleosomal DNA fragmentation and is useful for the detection of different stages of apoptosis in various cell types. Antibody reacts specifically with deoxycytidine and requires stretch of ssDNA of at least 25-30 bases in length for the binding. Importantly, in contrast with the TUNEL method, monoclonal antibodies to ssDNA are specific for apoptotic cell death and do not detect necrotic cells.

Species Cross-reactivity

All species

Immunogen

F7-26 was generated by the immunization of mice with calf thymus single-stranded DNA.

Method of Purification

Ammonium sulfate precipitation.

Storage and Handling

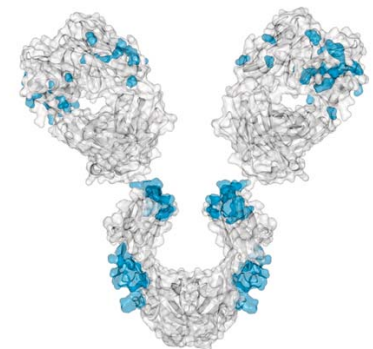
Stable for 1 years at -80°C in undiluted aliquots from date of receipt.

Control

Apoptotic cells.

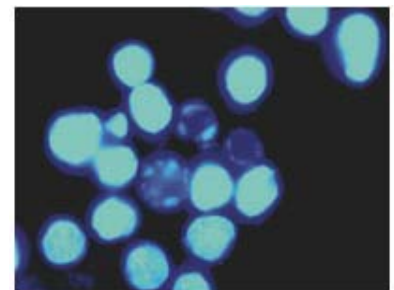
Research Applications

Immunohistochemistry: Formalin fixed, paraffin embedded tissues & 4% PF frozen tissues (special detection protocol required. FACS analysis (special protocol required).



References

1. Costourous J.G., et al. (2004). *J Orthop Res.* 22(3):678-683.
2. Tuder, R.M., et al. (2003). *Am J Respir Cell Mol. Biol.* 29:88-97.
3. Sivagurunathan S., et al. (2002). *Can. Res.* 62:3868-3875.
4. Gum J.R., et al. (2001). *Tumor Biol.* 61:3472-3479.
5. Studzinski D.M. and Benjamins JA. J. (2001). *Neuroscience Research.* 66:691-697.



Immunohistochemistry: Representative lot data. In cultures of breast cancer MB-MDA-468 cells treated with protein kinase inhibitor staurosporine, only cells with chromatin condensation typical of apoptosis were labeled with antibody to ssDNA (Catalog Number MAB3299).

APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry FC Flow Cytochemistry

IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry ELISA Enzyme-linked Immunosorbent Assay

SPECIES LEGEND: A All Species H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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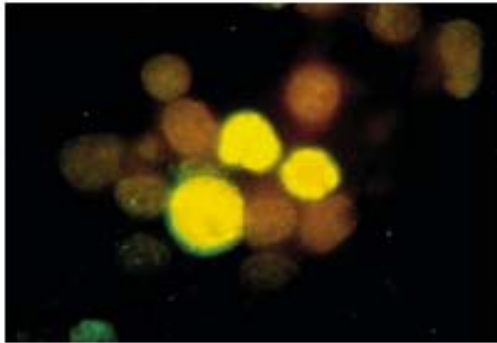


FIGURE 1. Representative lot data. Fluorescence microphotographs of MB-MDA-468 cells treated with staurosporine, heated in formamide, stained with MAB3299 (clone F7-26) and counterstained with DNA fluorochrome DAPI. The same field is shown after UV excitation for DAPI (left panel) and visible light excitation for fluorescein-labeled antibody (right panel). Note that only 3 apoptotic cells with chromatin condensed at the nuclear periphery are stained with the Mab. Magnification, x1000

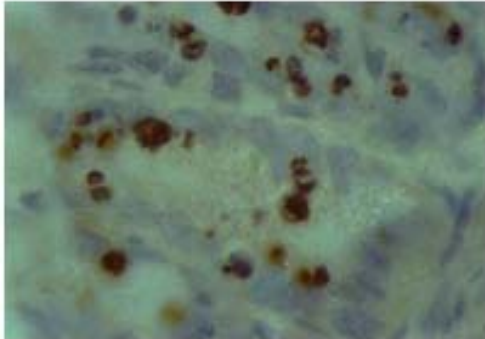


FIGURE 2. Representative lot data. Photomicrographs of small intestine crypts of a mouse treated with hydroxyurea (A) and small intestine crypts of a control animal (B), treated. Sections of formalin-fixed tissues were heated in formamide, stained with MAB3299 (Clone F7-26) and counterstained with hematoxylin. Brown nuclei - antibody stained apoptotic cells with condensed chromatin and fragmented nuclei.

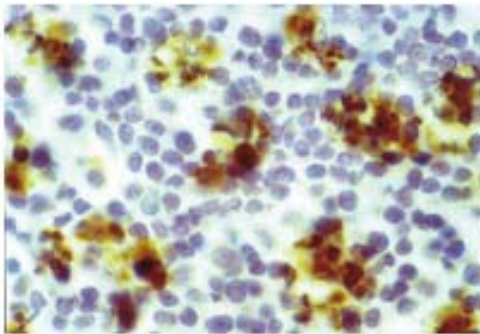


FIGURE 3. Representative lot data. Photomicrographs of thymus of a mouse treated with hydrocortisone (A) and thymus of a control animal (B). Sections of formalin-fixed tissues were heated in formamide, stained with MAB3299 (clone F7-26) and counterstained with hematoxylin. Brown nuclei - antibody stained apoptotic cells with condensed chromatin and fragmented nuclei.

Additional Research Applications

ELISA: 1:2000-1:5000 dilution from a previous lot worked.

Flow Cytometry: 1:00-1:200 dilution from a previous lot worked.

PROTOCOL

DETECTION PROTOCOLS: (NOTE: F7-26 requires a very specific protocols for successful use; the suggested protocol provided has been optimized for most situations).

A. Staining of tissue sections

Procedure includes the following steps:

Preparation of thin sections from **formalin** –fixed tissues using standard histological techniques. 2) Permeabilization in **saponin**. 3) Treatment with **Proteinase K**. 4) Heating in **formamide**. 5) Staining with anti-ssDNA monoclonal **antibody F7-26** and peroxidase conjugated **anti-mouse IgM**.

1. Fix tissues in 4% or 10% neutral buffered formalin or in **4% paraformaldehyde in PBS** at 4°C for 24 hours (no longer), dehydrate and embed in paraffin. **Important: fix in cold formalin for best results** (room temperature formalin gives higher backgrounds).
2. Cut 3-4 micron sections from paraffin blocks, attach sections to superfrost/plus slides and heat in oven at 56-60°C for 1-2 hours.
3. Deparaffinize tissue sections in xylene or in xylene substitute, wash sequentially in 100%, 95%, 70% ethanols and PBS.
4. Incubate slides in saponin (0.1 mg/mL in PBS) at room temperature for 20 min. Wash in PBS. *To prepare working concentration of saponin add 0.5 mL of stock solution (10 mg/ml saponin in distilled water) to 50 mL PBS.*
5. Incubate slides in Proteinase K (20 mg/mL in PBS) for 20 min at room temperature.
6. Wash slides in three changes of distilled water.
7. Transfer slides into coplin jar containing 50 ml of 50% formamide (v/v distilled H₂O) preheated in water bath to 56-60°C. Incubate slides in coplin jar with formamide for 20 min in water bath. **Important: temperature of formamide solution inside the jar should be 56° C**
8. After the heating, transfer slides into ice-cold PBS for 5 min.
9. Quench endogenous peroxidase in 3% hydrogen peroxide (freshly made) for 5 min. Rinse in distilled H₂O.
10. Treat sections with 3% non-fat dry milk for 15 min to block non-specific antibody binding. Rinse in PBS. *To prepare blocking solution dissolve non-fat dry milk powder in distilled water.*
11. Apply 100 microliters of monoclonal antibody F7-26 to the slide. Dilution is 1:10. Incubate at room temperature for 15 min and rinse in PBS. *To prepare working concentration of the antibody, add 4.5 mL of 1% non-fat dry milk in PBS to the vial containing 50 micrograms of F7-26 (0.5 mL). The diluted antibody should be aliquoted and stored frozen at -20°C. No more than 3 freeze thaws are recommended for F7-26.*
12. Apply 100 microliters of peroxidase-conjugated anti-mouse IgM, incubate 15 min and rinse with PBS. *To prepare working concentration of the antibody, dilute peroxidase-conjugated anti-mouse IgM in PBS {1:100-1:500}. Use only **freshly prepared** solution. We recommend peroxidase-conjugated anti-IgM because biotinavidin systems may produce non-specific cytoplasmic staining in some tissues. Signal intensity was strong without biotin-avidin amplification. Optimal working dilutions must be determined by end user.*
13. Apply chromagen solution (DAB), counterstain with hematoxylin and mount.

B. Staining of archival formalin-fixed tissues

The standard protocol described above provides optimal results after fixation in cold formalin or paraformaldehyde. Fixation at room temperature or prolong fixation may inhibit the staining, because excessive DNA-protein cross-links prevent formamide-induced DNA denaturation in apoptotic cells. Additional protease (pronase E) treatment is recommended for archival material if weak or negative staining is obtained with the standard protocol.

1. After Proteinase K treatment rinse slides in PBS and Tris buffer (10 mM, pH 7.6)
2. Incubate slides in Pronase E (20 microg/mL Tris buffer) at room temperature for 20 min in Coplin jar. (*Pronase E (Sigma, cat. #P6911) prepared as a stock solution 1 mg/ml in Tris buffer and stored frozen*)
3. Rinse slides in distilled water and proceed to the heating in formamide as in standard protocol.

C. Staining of Frozen tissue sections

1. Fix tissues in **cold** 4% paraformaldehyde in PBS for 18-24 hours (cold is best for low backgrounds).
2. Cut 4 micron sections, and immerse slides with sections into 85% methanol (6 parts of methanol and 1 part PBS) at room temperature for 30 minutes.
3. Air-dry fixed sections and store dried at room temperature.
4. Immerse slide with dry section into solution of Proteinase K (20 mg/mL in PBS); incubate at room temperature for 20 minutes.
5. Proceed as with paraffin sections (above) after the Proteinase K incubation (rinse with distilled water, heat in formamide etc.)

D. Control Procedures

The following control procedure is recommended to evaluate the specificity of Mab staining: Cell suspensions from drug-treated cultures, or tissue sections with high apoptotic indexes are heated and treated with S1 nuclease for 60 min at 37°C. S1 nuclease eliminates staining of apoptotic cells, demonstrating that Mab binds specifically to ssDNA. 1,000-2,000U/mL.

NOTE: Treatment of sections with commercial IgM as a negative control is not recommended because it may produce intensive background staining.

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E. Staining of cell suspensions for flow cytometry and fluorescence microscopy

Procedure includes the following steps:

Fixation in **methanol**, heating in **formamide**, and subsequent staining with anti-ssDNA monoclonal **antibody F7-26** and fluorescein-conjugated **anti-mouse IgM**.

1. Resuspend cell pellets in 1ml of cold PBS and slowly add 6 ml of methanol precooled to -20°C while vortexing.
2. Store fixed cells at -15 - 20°C for 1-3 days before staining.
3. Transfer 5×10^5 of fixed cells into 15 mL plastic tubes (Falcon 2096), centrifuge, remove supernatant.
4. Resuspend pellet in 0.25 mL of formamide. Keep 5 min at room temperature. Immerse rack with tubes into water bath preheated to 75 degrees C for 10 minutes. *Circulating water baths are recommended for optimal heating.* After the heating, immediately transfer rack with tubes into room temperature water.
5. Add 2 mL of 1% non-fat dry milk in PBS to the tubes containing formamide, vortex and keep tubes at room temperature for 15 min.
6. Centrifuge, remove supernatant, resuspend pellet in 100 microliters of monoclonal antibody F7-26. Antibody dilution is 1:10. Incubate at room temperature for 15 min. *To prepare working concentration of the antibody, add 4.5 mL of 5% fetal bovine serum in PBS to the vial containing 100 micro g of F7-26 (0.5mL). The diluted antibody could be aliquoted and stored frozen at -20°C . No more than 3 freeze-thaws are recommended for F7-26.*
7. Add 1 mL of PBS, centrifuge and resuspend pellet in 100 microliters of fluorescein-conjugated anti-mouse IgM. Incubate 15 min at room temperature. *To prepare working concentration of the antibody, dilute fluorescein-cojugated goat anti-mouse IgM 1:50 in 1% non-fat dry milk in PBS. The diluted antibody can be aliquoted and stored frozen at -20°C .*

For flow cytometry, add 1 mL of PBS, centrifuge and resuspend pellet in 0.5 mL of propidium iodide solution (1 mg/mL in PBS). For fluorescence microscopy, rinse cells with PBS, prepare cytopspins and stain slides with DNA fluorochrome DAPI (0.1 mg/mL in PBS for 10 min).

ADDITIONAL NOTES:

- Use only distilled water to diluted the formamide. No specific staining is observed with PBS/formamide.
- The Mg⁺⁺ ions used in the earlier protocols should not be included at any stage.
- Milk was found to be a more effective blocker than serum or simple BSA in most cases.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

RELATED PRODUCTS (specific)

cat #	description
MAB10229	■ Anti-DNA (Single Stranded), clone MRSS-1
CBL186	■ Anti-DNA, clone AC-30-10
MAB3032	■ Anti-DNA, double and single stranded, clone 844.2
MAB030	■ Anti-DNA, double stranded, 28% reactive with single stranded, clone BV16-13
MAB1293	■ Anti-DNA, double stranded, clone AE-2
MAB3868	■ Anti-DNA, single stranded
MAB3299	■ Anti-DNA, single stranded specific, clone F7-26
MAB10246	■ Anti-Double Stranded DNA, clone CH26-1352
S7821	■ CpGenome™ Universal Methylated DNA
S7822	■ CpGenome™ Universal Unmethylated DNA
17-316	■ DNA Replication Assay Kit

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 μm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 μm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 μm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0100	■ Immobilon Western Chemilum HRP Substrate 100 mL
17-373	■ Spray & Glow™ ECL WB Detection System 1 ea
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G

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