Product Datasheet

Separase Antibody (XJ11-4D7)
NB100-330

Unit Size: 0.1 ml

Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.

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Publications: 4

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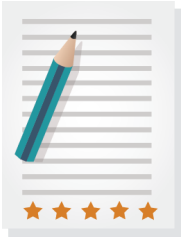
Updated 10/23/2024 v.20.1



NB100-330

Separase Antibody (XJ11-4D7)

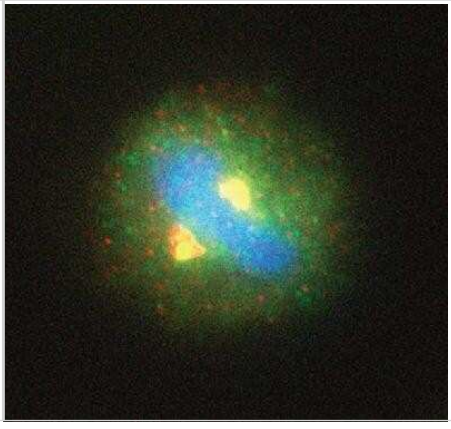
Product Information	
Unit Size	0.1 ml
Concentration	This product is unpurified. The exact concentration of antibody is not quantifiable.
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	XJ11-4D7
Preservative	0.1% Sodium Azide
Isotype	IgG2a
Purity	Unpurified
Buffer	Ascites

Product Description	
Host	Mouse
Gene ID	9700
Gene Symbol	ESPL1
Species	Human, Mouse
Reactivity Notes	Use in Mouse reported in scientific literature (PMID:17626243).
Immunogen	Maltose-Binding Protein fusion of C-terminal fragment of human Separase (amino acids 1866-1996). [UniProt# Q14674]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot reported in scientific literature (PMID 17626243), Immunocytochemistry/ Immunofluorescence 1:100-1:1000
Application Notes	In ICC/IF, this antibody recognizes endogenous Separase in paraformaldehyde-fixated preparations of human cells.

Images

Immunocytochemistry/Immunofluorescence: Separase Antibody (XJ11-4D7) [NB100-330] - Overlay [blue] of centrosomal staining in HeLa cells. Centrosomal staining of separase [yellow], using NB 100-330, and gamma tubulin [green] in mitotic-metaphase cells and nuclear staining of separase in pre-mitotic cells.



Publications

Sak A, Fegers I, Groneberg M Effect of separase depletion on ionizing radiation-induced cell cycle checkpoints and survival in human lung cancer cell lines. Cell Prolif. 2008-08-01 [PMID: 18616699] (WB, Human)

Rubinek T, Chesnokova V, Wolf I et al. Discordant Proliferation and Differentiation in Pituitary Tumor-Transforming Gene-Null Bone Marrow Stem Cells Am J Physiol Cell Physiol. 2007-09-01 [PMID: 17626243] (WB, Mouse)

Fujita T, Epperly M, Zou H et al. Regulation of the anaphase-promoting complex-separase cascade by transforming growth factor-beta modulates mitotic progression in bone marrow stromal cells. Mol Biol Cell. 2008-12-01 [PMID: 18843049] (WB, Human)

Kim H, Jeon Y, Ha G et al. Functional interaction between BubR1 and securin in an anaphase-promoting complex/cyclosomeCdc20-independent manner. Cancer Res. 2009-01-01 [PMID: 19117984] (WB, Human)

Procedures

Immunocytochemistry/Immunofluorescence protocol for Separase Antibody (NB100-330)
Separase Antibody (XJ11-4D7):
Immunofluorescence Procedure Cell Preparation
1. HeLa cells are grown on coverslips and enriched in a fraction of mitotic cells by double thymidine block/release protocol. 2. Briefly, cells are allowed to attach to the glass coverslips for 16 hours. 3. Thymidine is added to the culturing medium at a final concentration of 2 mM for 18 hours. 4. Cells are washed 2 times with PBS. 5. Fresh medium without thymidine is added. 6. Cells are incubated in the thymidine-free medium for 8 hours. 7. Thymidine is added again to a final 2 mM concentration for 18 hours again. 8. After the second thymidine incubation, the cells are washed 2 times with PBS. 9. Fresh thymidine-free medium is added again. 10 .Starting at 8 hours after the second thymidine removal, cells are analyzed by light microscopy and once population of mitotic cells are about 50% (usually around 9-10 hours after the second release) the cell staining procedure begins.
Cell Staining 1. Cells are washed 2 times with Dulbecco's PBS with Ca and Mg (D-PBS) and permeabilized prior to fixation by incubation with 0.05% Triton X-100 for 1 min. 2. Permeabilizing solution is aspirated and 4% paraformaldehyde is added for 30-45 min. 3. Paraformaldehyde is removed by washing the coverslips 3 times with D-PBS. 4. Cells are blocked by SuperBlock reagent (Pierce) in PBS supplemented with 0.5% Triton X-100 for 30 min. 5. The coverslips are washed 3 times with 0.05% Tween-20 in PBS (PBS-T). 6. Staining to detect separase is carried out at a dilution of 1:1,000 of mouse monoclonal anti-separase (NB 100-330) prepared in PBS-T and incubated with the cells on coverslips for 1 hour at room temperature (RT). 7. Coverslips are washed 3 x 5 min. washes with PBS-T. 8. Incubate cells with an anti-mouse Cy3 conjugated fluorochrome-labeled secondary. The secondary antibody is diluted in PBS-T and incubated for 20 min. 9. Coverslips are incubated with DAPI at a final concentration of 10 ng/ml in PBS-T for 5 min. 10.Cells are washed 5 x 5 min with PBS-T. 11.Prior to mounting, the coverslips are washed once with PBS without detergents. 12. Micrographs are taken by a fluorescent microscope.

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Products Related to NB100-330

HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)
BC110-55296PEP	Separase Antibody Blocking Peptide

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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