

# Product Datasheet

## Separase Antibody (XJ11-4D7) - BSA Free NB100-330

Unit Size: 0.1 ml

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

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[technical@novusbio.com](mailto:technical@novusbio.com)

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**NB100-330**

Separase Antibody (XJ11-4D7) - BSA Free

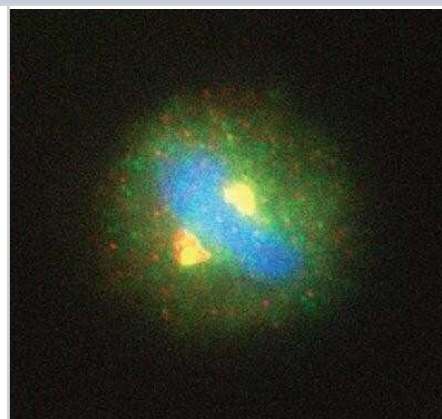
Product Information	
<b>Unit Size</b>	0.1 ml
<b>Concentration</b>	This product is unpurified. The exact concentration of antibody is not quantifiable.
<b>Storage</b>	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	XJ11-4D7
<b>Preservative</b>	0.1%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

Product Description	
<b>Description</b>	Novus Biologicals Mouse Separase Antibody (XJ11-4D7) - BSA Free (NB100-330) is a monoclonal antibody validated for use in WB and ICC/IF. Anti-Separase Antibody: Cited in 4 publications. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Mouse
<b>Gene ID</b>	9700
<b>Gene Symbol</b>	ESPL1
<b>Species</b>	Human, Mouse
<b>Reactivity Notes</b>	Use in Mouse reported in scientific literature (PMID:17626243).
<b>Immunogen</b>	Maltose-Binding Protein fusion of C-terminal fragment of human Separase (amino acids 1866-1996). [UniProt# Q14674]

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





### **Novus Biologicals USA**

10730 E. Briarwood Avenue  
Centennial, CO 80112  
USA  
Phone: 303.730.1950  
Toll Free: 1.888.506.6887  
Fax: 303.730.1966  
nb-customerservice@bio-techne.com

### **Bio-Techne Canada**

21 Canmotor Ave  
Toronto, ON M8Z 4E6  
Canada  
Phone: 905.827.6400  
Toll Free: 855.668.8722  
Fax: 905.827.6402  
canada.inquires@bio-techne.com

### **Bio-Techne Ltd**

19 Barton Lane  
Abingdon Science Park  
Abingdon, OX14 3NB, United Kingdom  
Phone: (44) (0) 1235 529449  
Free Phone: 0800 37 34 15  
Fax: (44) (0) 1235 533420  
info.EMEA@bio-techne.com

### **General Contact Information**

www.novusbio.com  
Technical Support: nb-technical@bio-techne.com  
Orders: nb-customerservice@bio-techne.com  
General: novus@novusbio.com

### **Products Related to NB100-330**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-96778	Mouse IgG2a Isotype Control (M2A)

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### **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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