

# Product Datasheet

## Ceramide Kinase Antibody - BSA Free NB100-2911

Unit Size: 0.1 ml

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

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**NB100-2911**

Ceramide Kinase Antibody - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	0.82 mg/ml
Storage	Aliquot and store at -20C or -80C. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Glycine and 0.15M NaCl
Target Molecular Weight	65 kDa

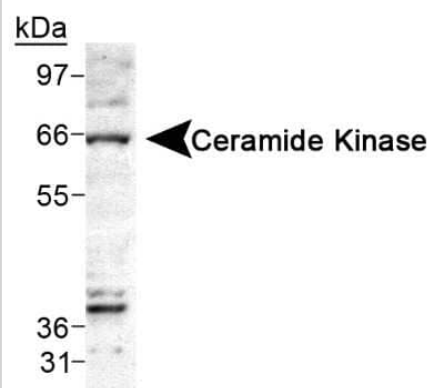
Product Description	
Host	Rabbit
Gene ID	64781
Gene Symbol	CERK
Species	Human, Mouse
Immunogen	A synthetic peptide made to an internal portion of human Ceramide Kinase (between residues 50-150). [Swiss-Prot# Q8TCT0]

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 2 ug/ml, Immunocytochemistry/ Immunofluorescence 1:500-1:2000
Application Notes	This Ceramide Kinase antibody is useful for Western Blot and Immunocytochemistry/Immunofluorescence. In Western blot a band is seen at ~65 kDa representing the Ceramide Kinase protein, and in Immunocytochemistry/Immunofluorescence vesicular cytoplasmic staining was observed in A431 cells.

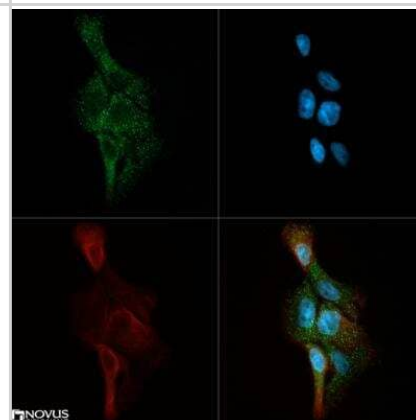


## Images

Western Blot: Ceramide Kinase Antibody [NB100-2911] - Detection of Ceramide Kinase in A549 cell lysate using NB100-2911.



Immunocytochemistry/Immunofluorescence: Ceramide Kinase Antibody [NB100-2911] - Ceramide Kinase antibody was tested in A431 cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).



## Publications

Tian L, Zhao C, Yan Y et al. Ceramide-1-phosphate alleviates high-altitude pulmonary edema by stabilizing circadian ARNTL-mediated mitochondrial dynamics Journal of advanced research 2023-07-20 [PMID: 37479181] (WB, Mouse)

Bhadwal P, Randhawa V, Vaiphei K et al. Clinical relevance of CERK and SPHK1 in breast cancer and their association with metastasis and drug resistance Scientific reports 2022-10-29 [PMID: 36309544] (WB, Human)

Details:

Dilution used in WB 1:1000

Doria M Luisa, Ribeiro Ana S, Wang Jun et al. Fatty acid and phospholipid biosynthetic pathways are regulated throughout mammary epithelial cell differentiation and correlate to breast cancer survival. FASEB J. 2014-06-26 [PMID: 24970396] (WB, Mouse)

## Procedures

### Western Blot protocol for Ceramide Kinase Antibody (NB100-2911)

Ceramide Kinase Antibody:

Procedure Guide for NB 100-2911 - Ceramide Kinase Antibody

#### Western Blot Protocol

1. Perform SDS-PAGE (4-12%) on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to Nitrocellulose according to the instructions provided by the manufacturer of the transfer apparatus.
3. Rinse membrane with dH<sub>2</sub>O and then stain the blot using ponceau S for 1-2 minutes to access the transfer of proteins onto the nitrocellulose membrane. Rinse the blot in water to remove excess stain and mark the lane locations and locations of molecular weight markers using a pencil.
4. Rinse the blot in TBS for approximately 5 minutes.
5. Block the membrane using 5% non-fat dry milk + 1% BSA in TBS overnight at 4 degrees Celcius.
6. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
7. Dilute the rabbit anti-Ceramide kinase primary antibody (NB 100-2911) in blocking buffer and incubate 1 hour at RT.
8. Rinse the membrane in dH<sub>2</sub>O and then wash the membrane in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each.
9. Apply the diluted rabbit-IgG HRP-conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer [TBS + 0.1% Tween] 3 times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions (we used BioFX Super Plus ECL).

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%, provided it does not interfere with antibody-antigen binding.

### Immunocytochemistry/Immunofluorescence protocol for Ceramide Kinase Antibody (NB100-2911)

Ceramide Kinase Antibody:

Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

\*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.



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### **Products Related to NB100-2911**

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NB100-2911PEP	Ceramide Kinase Antibody Blocking Peptide
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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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