Product Datasheet

PKC zeta Antibody (R14-K) NBP1-30127-0.05ml

Unit Size: 0.05 ml

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.



Publications: 1

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NBP1-30127-0.05ml

PKC zeta Antibody (R14-K)

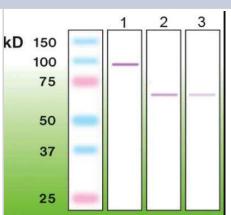
Product Information	
Unit Size	0.05 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	R14-K
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	20mM Tris/HCI (pH 8.0) and 10 mg/ml BSA
Product Description	
Description	This antibody is immunoaffinity purified with immunogenic peptide as a ligand.
Host	Rabbit
Gene ID	5590
Gene Symbol	PRKCZ
Species	Human, Mouse, Rat
Specificity/Sensitivity	This antibody is specific for PKC-Zeta.
Immunogen	Peptide derived from internal sequence on human PKC zeta. Antibody recognizes the epitope located between Lys220 - Leu231
Notes	This antibody is immunoaffinity purified with immunogenic peptide as a ligand.
Product Application Details	
Applications	Western Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation
Recommended Dilutions	Western Blot 1:5000, ELISA 1:20000-1:100000, Immunocytochemistry/ Immunofluorescence 1:10-1:500, Immunoprecipitation
Application Notes	Dilutions for IP must be determined by end user.

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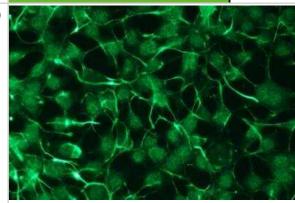


Images

Western Blot: PKC zeta Antibody (R14-K) [NBP1-30127] - Lane 1-50ng of recombinant human PKC-zeta (N-term GST PKC-zeta; BML-SE443-0005); lane 2-mouse brain crude protein lysate (100 ug); lane 3-rat brain crude.



Immunocytochemistry/Immunofluorescence: PKC zeta Antibody (R14-K) [NBP1-30127] - Expression in HEK293 cells.





Procedures

Immunoprecipitation (IP) protocol

1. Dilute sample (200 - 500 ug of total protein) with Correction Buffer (2.5% v/v Nonidet P-40, 5% w/v sodium deoxycholate, 0.5% w/v SDS in ddH2O) in ratio 4:1 (v/v).

2. Add 5 ul of the antibody, mix gently and incubate for 1 hour on ice.

3. Mix with 50 ul of ProteinG-Sepharose (washed with 10mM Tris-HCl, pH7.5*) and incubate for 30 minutes at 4 degrees C with gentle shaking.

a. *NOTE: Washing of ProteinG-Sepharose with 10mM Tris-HCl, pH7.5: Resuspend 50 ul of ProteinG-Sepharose in 1 ml of 10mM Tris-HCl, pH7.5 by precise inverting of the tube several times. Centrifuge for 1 min, 900xg at 4 degrees C and discard supernatant. Avoid of wasting ProteinG-Sepharose agarose gel beads during discarding. Repeat this procedure for 3 times.

4. Centrifuge ProteinG-Sepharose immunocomplex for 2 min, 900xg at 4 degrees C and discard supernatant. (DO NOT spin any faster as this can cause the binding affinity to decrease).

5. Wash the pellet 3 times with 1 ml of RIPA Buffer (10mM TRIS-HCl, pH7.5, 140mM NaCl, 1% v/v Nonidet P-40, 1% w/v sodium deoxycholate, 0.5% w/v SDS v ddH2O).

a. IMPORTANT: Avoid of wasting/discarding ProteinG-Sepharose immunocomplex.

6. Wash sediment with 1 ml of 10mM TRIS-HCl, pH7.5, centrifuge sample (agarose gel beads) for 1 min, 900xg at 4 degrees C and discard the supernatant.

7. Dissociate immunocomplex from ProteinG-Sepharose with the help of Reduction Buffer (125mM TRIS- HCl, pH6.8, 3.3% SDS, 5% beta-mercaptoethanol). Mix the sample with 30 ul of Reduction Buffer, shake gently and incubate for 5 minutes at 65 degrees C.

8. Centrifuge at 3000xg for 5 minutes and transfer the supernatant (immunoprecipitated proteins) to new tube.

9. Separate the immunoprecipitated protein by 1D SDS-PAGE





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Products Related to NBP1-30127-0.05ml

NB820-59182	Human Brain Cerebral Cortex Whole Tissue Lysate (Adult Whole Normal)
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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