Product Datasheet

14-3-3 gamma [ac Val2] Antibody (KC21) - BSA Free
NB100-406

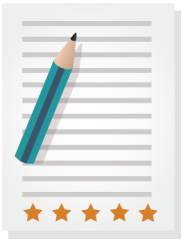
Unit Size: 0.1 ml

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

www.novusbio.com technical@novusbio.com

Protocols, Publications, Related Products, Reviews, Research Tools and Images at:
www.novusbio.com/NB100-406

Updated 4/13/2025 v.20.1



NB100-406

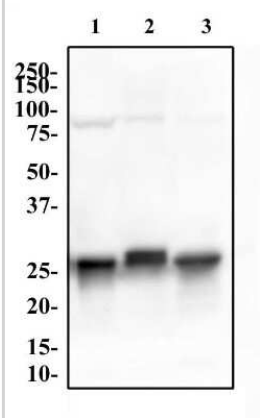
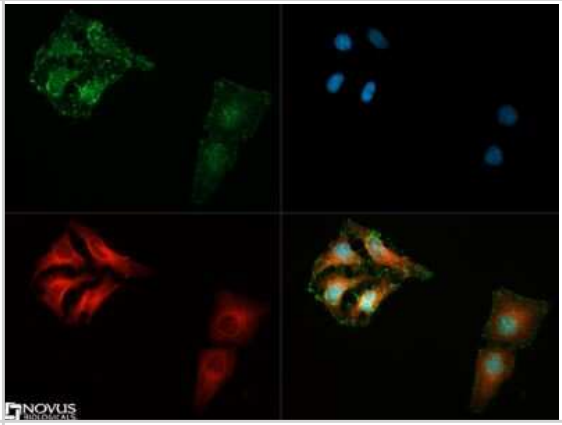
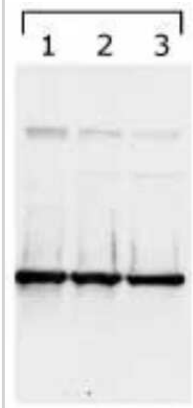
14-3-3 gamma [ac Val2] Antibody (KC21) - BSA Free

Product Information	
Unit Size	0.1 ml
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	KC21
Preservative	0.02% Sodium Azide
Isotype	IgG2a
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	33 kDa

Product Description	
Host	Mouse
Gene ID	7532
Gene Symbol	YWHAG
Species	Human
Specificity/Sensitivity	This antibody is specific for the naturally occurring human form of 14-3-3 gamma, where the N-terminal Met is removed, resulting in an acetylated N-terminal Val. Unprocessed (non-modified) 14-3-3 gamma is not recognized by this antibody.
Immunogen	N-terminal fragment of human 14-3-3 gamma where the N-terminal Val was acetylated [UniProt# P61981]

Product Application Details	
Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence
Recommended Dilutions	Western Blot 1:1000-1:3000, Simple Western 1:12.5, Immunocytochemistry/ Immunofluorescence 1:500

Images

Western Blot: 14-3-3 gamma [ac Val2] Antibody (KC21) [NB100-406] - (1) HeLa, (2) PC12 and (3) NIH-3T3 whole cell lysates were separated by SDS-PAGE on a 12% gel and transferred to PVDF membrane. The membrane was probed with Anti-14-3-3 gamma [Ac Val2] antibody at 2 ug/ml for 1 hour, followed by incubation a 1:10,000 dilution of anti-rabbit HRP. The protein was detected at approximately 28 kDa after incubation with SuperSignal West Pico Chemiluminescent substrate.	
Immunocytochemistry/Immunofluorescence: 14-3-3 gamma [ac Val2] Antibody (KC21) [NB100-406] - 14-3-3 gamma [ac Val2] antibody was tested in SH-SY5Y cells with DyLight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and DyLight 550 (red).	
Western Blot: 14-3-3 gamma [ac Val2] Antibody (KC21) [NB100-406] - 14-3-3 gamma detected in lysates using NB100-406. Lane 1: HeLa cell lysates, Lanes 2 and 3: bengamide treated lysates (8h and 24h, respectively).	
Simple Western: 14-3-3 gamma [ac Val2] Antibody (KC21) [NB100-406] - Lane view shows a specific band for 14-3-3 Gamma in 1.0 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230kDa separation system.	

Procedures

Western Blot protocol for 14-3-3 gamma Antibody (NB100-406)
14-3-3 gamma [ac Val2] Antibody (KC21):
1. Load protein on gel (ie: ~30 ug of HeLa whole cell control lysate) and run.
2. Transfer protein to nitrocellulose (Schleicher&Schuell, cat# BA83).
3. Block the membrane with 5% milk for 30 minutes at room temperature.
4. Incubate the membrane with anti-14-3-3 gamma [cat# NB 100-406] (1:1,000), overnight at 4C or 2 h at room temperature. Milk or PBS-T are good for this dilution.
5. Wash the membrane 3 times, 10 minutes per wash in PBS with 0.1% Tween-20 (PBS-T).
6. Incubate with secondary antibody for 30 minutes at room temperature. Use milk in PBS-T as a diluent.
7. Rinse the membrane 2 times with deionized water and place in an ECL working solution.
8. Expose to the film.

Immunocytochemistry/ Immunofluorescence Protocol for 14-3-3 gamma Antibody (NB100-406)
14-3-3 gamma [ac Val2] Antibody (KC21):
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 1:25,0000 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.

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 NB100-406

NB800-PC1	HeLa Whole Cell Lysate
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)

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.

For more information on our 100% guarantee, please visit www.novusbio.com/guarantee

Earn gift cards/discounts by submitting a review: www.novusbio.com/reviews/submit/NB100-406

Earn gift cards/discounts by submitting a publication using this product: www.novusbio.com/publications