abcam

## Product datasheet

# Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free ab227987

KO VALIDATED Recombinant RabMAb

9 References 13 Images

### Overview

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|---|---|
| **Product name** | Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR2663] to TNFAIP3 - BSA and Azide free |
| **Host species** | Rabbit |
| **Specificity** | **Mouse species is recommended based on WB results, we do not guarantee IHC-P for mouse.** |
| **Tested applications** | **Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF |
| **Species reactivity** | **Reacts with:** Mouse, Human |
| **Immunogen** | Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. |
| **Positive control** | WB: WEHI-3 treated with TNF (ab9642), Jurkat treated with TNF (ab9642) + TPA, Jurkat treated with 5ng/ml PMA for 48 hours and then treated with 2Âµg/ml PHA for 48 hours, HeLa, A549 and Daudi cell lysates. IHC-P: Human kidney tissue. ICC/IF: Daudi and HeLa cells. Flow Cyt (intra): HepG2 and Daudi cells. |
| **General notes** | ab227987 is the carrier-free version of ab92324.<br><br>Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.<br><br>Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.<br><br>This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. |

### Properties

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| **Form** | Liquid |
| **Storage instructions** | Shipped at 4°C. Store at +4°C. Do Not Freeze. |
| **Storage buffer** | pH: 7.20<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR2663 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our Abpromise guarantee covers the use of ab227987 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

| Application | Abreviews | Notes |
|---|---|---|
| **Flow Cyt (Intra)** | | Use at an assay dependent concentration.<br>ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody. |
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 90 kDa. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.<br>**Mouse species is recommended based on WB results, we do not guarantee IHC-P for mouse.** |
| **ICC/IF** | | Use at an assay dependent concentration. |

## Target

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| **Function** | Ubiquitin-editing enzyme that contains both ubiquitin ligase and deubiquitinase activities. Essential component of a ubiquitin-editing protein complex, comprising also RNF11, ITCH and TAX1BP1, that ensures the transient nature of inflammatory signaling pathways. Upon TNF stimulation, deubiquitinates 'Lys-63'-polyubiquitin chains on RIPK1 and catalyzes the formation of 'Lys-48'-polyubiquitin chains. This leads to RIPK1 proteasomal degradation and consequently termination of the TNF- or LPS-mediated activation of NF-kappa-B. In vitro able to deubiquitinate both 'Lys-48'- and 'Lys-63' polyubiquitin chains. Inhibitor of programmed cell death. Has a role in the function of the lymphoid system. |
| **Sequence similarities** | Belongs to the peptidase C64 family.<br>Contains 7 A20-type zinc fingers.<br>Contains 1 OTU domain. |
| **Domain** | The A20-type zinc fingers mediate the ubiquitin ligase activity.<br>The OTU domain mediates the deubiquitinase activity. |

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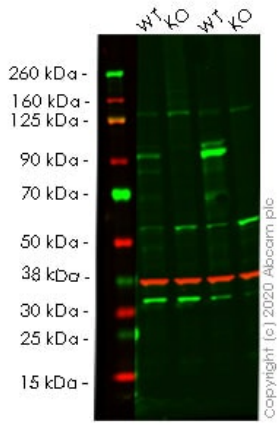
|---|---|
| **Cellular localization** | Cytoplasm. Nucleus. |

## Images




Western blot - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

**All lanes :** Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

**Lane 1 :** Wild-type A549 cell lysate
**Lane 2 :** TNFAIP3 knockout A549 cell lysate
**Lane 3 :** Wild-type HeLa cell lysate
**Lane 4 :** TNFAIP3 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

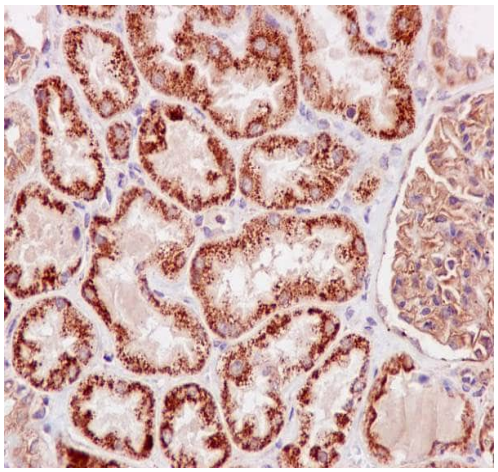
Performed under reducing conditions.

**Predicted band size:** 90 kDa
**Observed band size:** 90 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab92324).

**Lanes 1- 4:** Merged signal (red and green). Green - ab92324 observed at 90 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

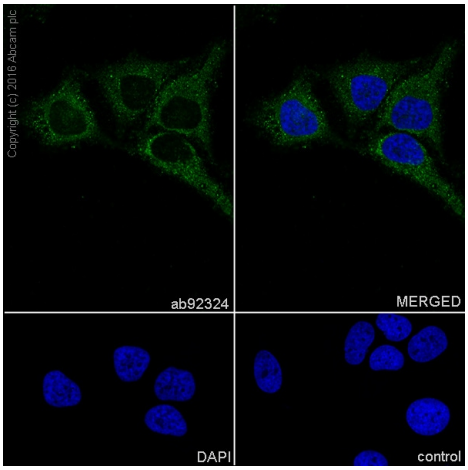
ab92324 was shown to react with TNFAIP3 in wild-type A549 cells in western blot. Loss of signal was observed when knockout cell line ab266946 (knockout cell lysate ab257114) was used. Wild-type A549 and TNFAIP3 knockout A549 cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling TNFAIP3 with purified ab92324 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.

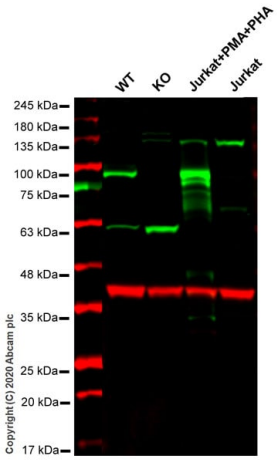
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).




Immunocytochemistry/ Immunofluorescence - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labelling TNFAIP with purified ab92324 at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% triton X-100. ab150077 Goat anti rabbit IgG (Alexa Fluor® 488) at 1/1000 was used as the secondary antibody. Nuclei were counterstained with DAPI. PBS was used instead of the primary antibody as the negative control.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).




Western blot - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

**All lanes :** Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate
**Lane 2 :** TNFAIP3 knockout HeLa cell lysate
**Lane 3 :** Jurkat cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate
**Lane 4 :** Untreated Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

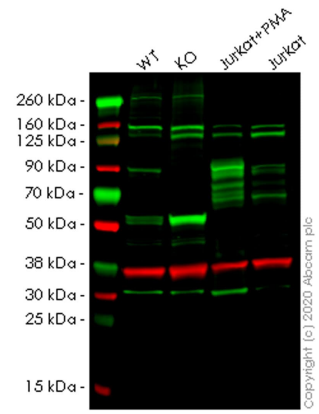
**Predicted band size:** 90 kDa
**Observed band size:** 80 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab92324).

**Lanes 1-4:** Merged signal (red and green). Green - ab92324 observed at 80 kDa. Red - loading control, ab8245 observed at 37 kDa.

ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265983 (knockout cell lysate ab257112) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature

before imaging.




Western blot - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

**All lanes :** Anti-TNFAIP3 antibody [EPR2663] (ab92324) at 1/1000 dilution

**Lane 1 :** Wild-type A549 (Human lung carcinoma cell line) whole cell lysate
**Lane 2 :** TNFAIP3 knockout A549 (Human lung carcinoma cell line) whole cell lysate
**Lane 3 :** Jurkat (Human T cell leukemia cell line from peripheral blood) cell treated with 5ng/ml PMA for 48 hours and then treated with 2µg/ml PHA for 48 hours, whole cell lysate
**Lane 4 :** Untreated Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

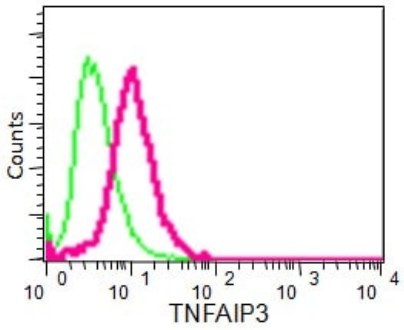
**Predicted band size:** 90 kDa
**Observed band size:** 80 kDa

This data was developed using ab92324, the same antibody clone in a different buffer formulation.

**Lanes 1-4:** Merged signal (red and green). Green - ab92324 observed at 80 kDa. Red - loading control ab8245 observed at 36 kDa.

ab92324 Anti-TNFAIP3 antibody [EPR2663] was shown to specifically react with TNFAIP3 in wild-type A549 cells. Loss of signal was observed when knockout cell line ab266945 (knockout cell lysate ab257113) was used. Wild-type and TNFAIP3 knockout samples were subjected to SDS-PAGE. ab92324 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary

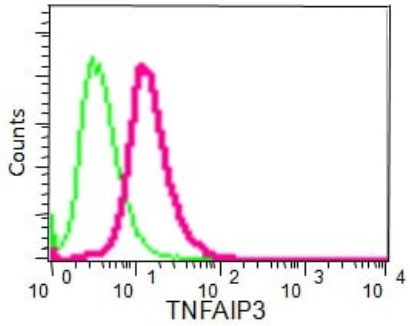
antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.




Flow Cytometry (Intracellular) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Intracellular Flow Cytometry analysis of Daudi cells labelling TNFAIP3 with purified ab92324 at 1/30 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.

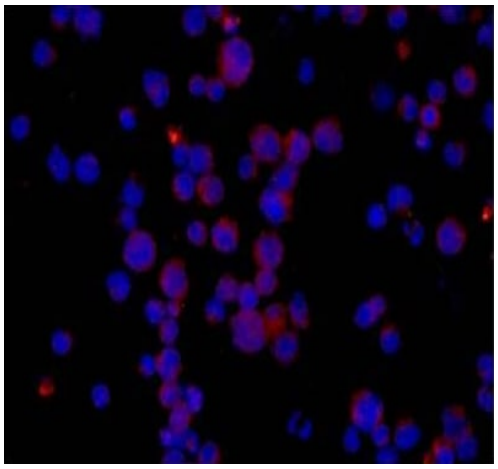
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).




Flow Cytometry (Intracellular) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Intracellular Flow Cytometry analysis of Daudi cells labelling TNFAIP3 with unpurified ab92324 at 1/20 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Green - Isotype control, rabbit monoclonal IgG.

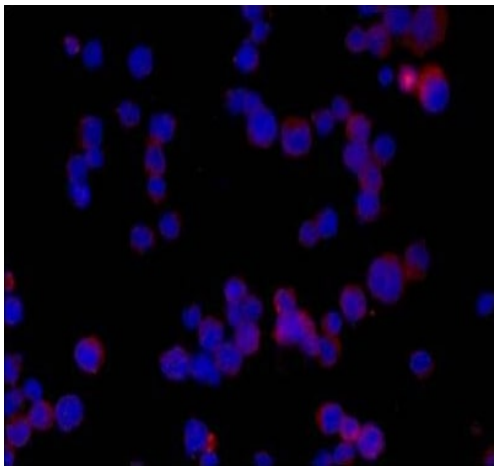
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).



Immunocytochemistry/ Immunofluorescence - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Immunocytochemsitry/Immunofluorescence analysis of Daudi cells labelling TNFAIP3 (red) with purified ab92324 at 1/100. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).

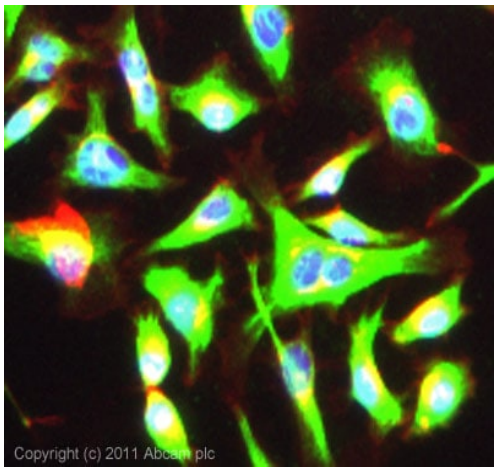
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).



Immunocytochemistry/ Immunofluorescence - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Immunocytochemsitry/Immunofluorescence analysis of Daudi cells labelling TNFAIP3 (red) with unpurified ab92324 at 1/50. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. Counterstained with DAPI (blue).

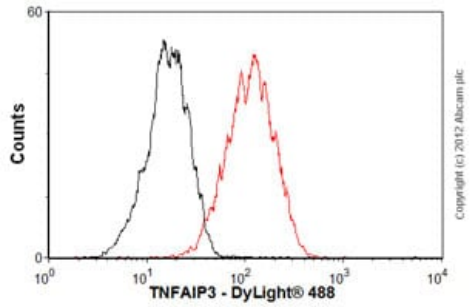
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).




Immunocytochemistry/ Immunofluorescence - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

ICC/IF image of unpurified ab92324 stained HeLa cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab92324, neat) overnight at +4°C. The secondary antibody (green) was ab96899 Dylight 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

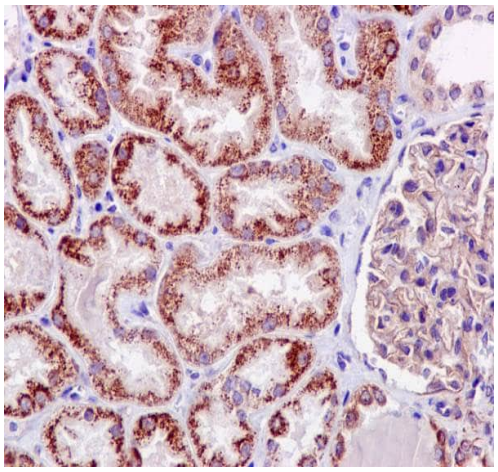
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).




Flow Cytometry (Intracellular) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

Overlay histogram showing HepG2 cells stained with unpurified ab92324 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab92324, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HepG2 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

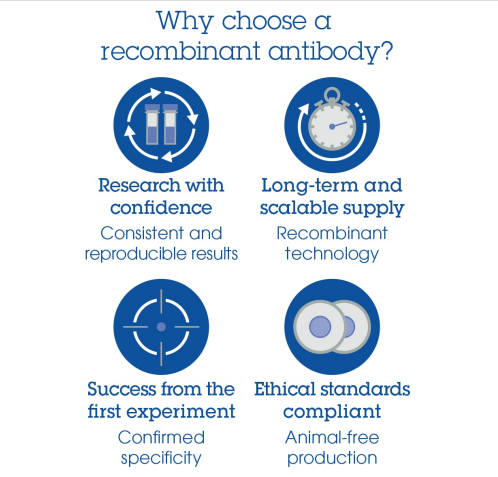
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab92324).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)

This IHC data was generated using the same anti-TNFAIP3 antibody clone, EPR2663, in a different buffer formulation (cat# ab92324).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling TNFAIP3 with unpurified ab92324 at 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with Hematoxylin.




Anti-TNFAIP3 antibody [EPR2663] - BSA and Azide free (ab227987)



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