Product Datasheet

mtTFA Antibody (18G102B2E11) - BSA Free
NBP1-71648SS

Unit Size: 0.025 ml

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

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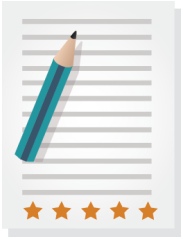
Updated 4/22/2025 v.20.1



NBP1-71648SS

mtTFA Antibody (18G102B2E11) - BSA Free

Product Information

Unit Size	0.025 ml
Concentration	1 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	18G102B2E11
Preservative	0.05% Sodium Azide
Isotype	IgG2b Kappa
Purity	Protein G purified
Buffer	Tris-Glycine and 0.15M NaCl

Product Description

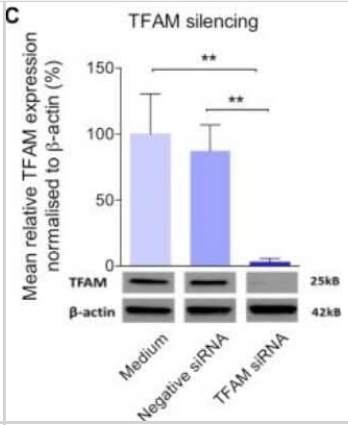
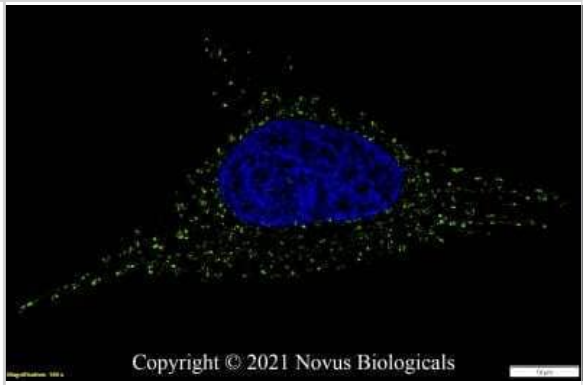
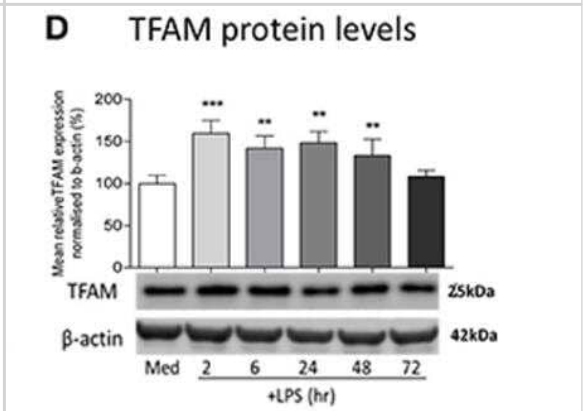
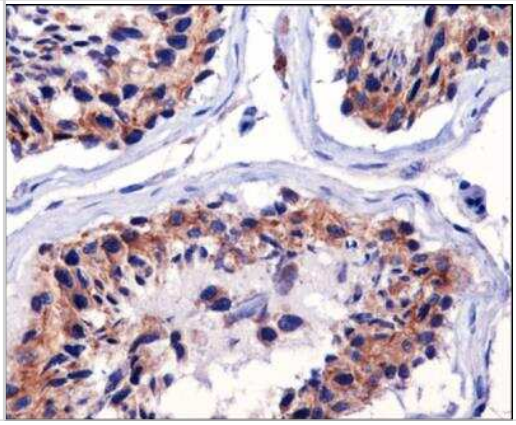
Host	Mouse
Gene ID	7019
Gene Symbol	TFAM
Species	Human, Mouse, Rat
Reactivity Notes	Mouse reactivity reported in scientific literature (PMID: 28053692)
Immunogen	Human mtTFA [Swiss-Prot# Q00059]

Product Application Details

Applications	Western Blot, Simple Western, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Knockdown Validated, Knockout Validated
Recommended Dilutions	Western Blot 1:1000, Simple Western 1:20, Immunohistochemistry 1:200, Immunocytochemistry/ Immunofluorescence 1-5 ug/ml, Immunohistochemistry-Paraffin 1:200, Knockout Validated reported in scientific literature (PMID 32001952), Knockdown Validated
Application Notes	In Western blot, a band can be seen around 20 - 25 kDa. In Simple Western only 10 - 15 uL of the recommended dilution is used per data point. See Simple Western Antibody Database for Simple Western validation: Tested in HeLa lysate 0.5 mg/mL, separated by Size, antibody dilution of 1:20, apparent MW was 33 kDa. Separated by Size-Wes, Sally Sue/Peggy Sue.

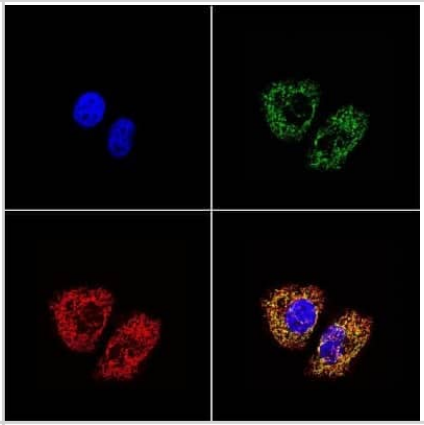
Images

Simple Western: mtTFA Antibody (18G102B2E11) [NBP1-71648] - Simple Western lane view shows a specific band for mtTFA in 0.5 mg/ml of HeLa lysate. This experiment was performed under reducing conditions using the 12-230 kDa separation system.

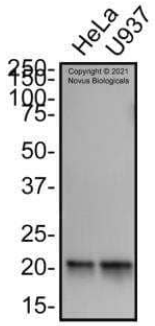
mtTFA-Antibody-18G102B2E11-Western-Blot-NBP1-71648-img0010.jpg	
Immunocytochemistry/Immunofluorescence: mtTFA Antibody (18G102B2E11) [NBP1-71648] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-mtTFA Antibody (18G102B2E11) NBP1-71648 at 1 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 100X objective and digitally deconvolved.	
mtTFA-Antibody-18G102B2E11-Western-Blot-NBP1-71648-img0013.jpg	
Immunohistochemistry-Paraffin: mtTFA Antibody (18G102B2E11) [NBP1-71648] - IHC staining of mtTFA on human testis using DAB with hematoxylin counterstain.	

Immunocytochemistry/Immunofluorescence: mtTFA Antibody (18G102B2E11) [NBP1-71648] - HeLa cells were fixed in 4% paraformaldehyde for 10 minutes and permeabilized in 0.05% Triton X-100 in PBS for 5 minutes. The cells were incubated with anti-mtTFA Antibody (18G102B2E11) NBP1-71648 at 1 ug/ml overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:1000 dilution for 60 minutes. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.

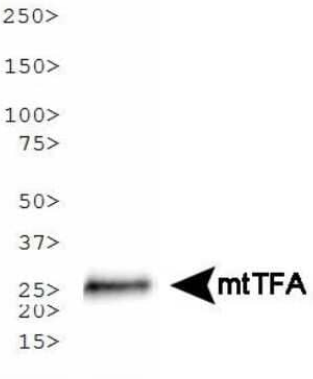
Immunocytochemistry/Immunofluorescence: mtTFA Antibody (18G102B2E11) [NBP1-71648] - Immunostaining in HeLa cells. C1: DAPI staining 2 nuclei, C2: anti-mtTFA stain with Alexa Fluor 488 secondary ab (green), C3: Mitotracker Red staining mitochondria. Image courtesy of Elizabeth Wang.



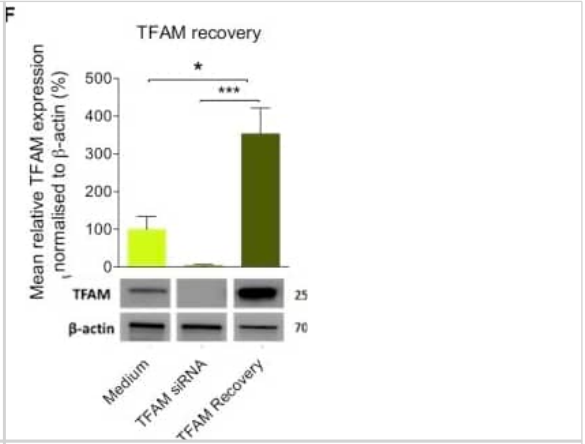
Western Blot: mtTFA Antibody (18G102B2E11) [NBP1-71648] - Total protein from Hela and U937 were separated on a 12% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% non-fat milk in TBST. The membrane was probed with 2.0 ug/ml anti-mtTFA NBP1-71648 in blocking buffer and detected with an anti-mouse HRP secondary antibody using NovaLume chemiluminescence detection reagent (NPB2-61915).



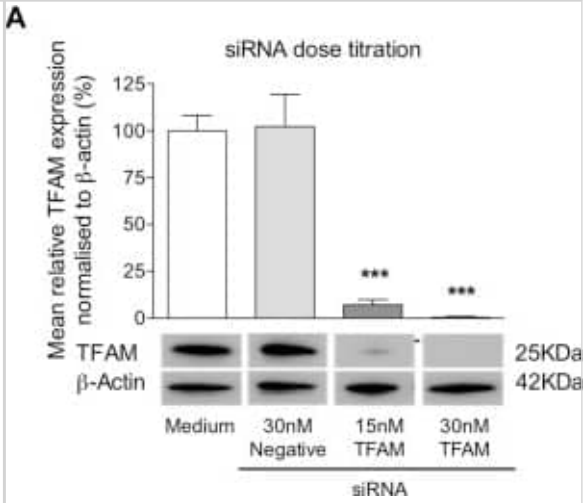
Western Blot: mtTFA Antibody (18G102B2E11) [NBP1-71648] - Analysis of mtTFA expression in HeLa whole cell lysate.



Western Blot: mtTFA Antibody (18G102B2E11) [NBP1-71648] - MtDNA depletion & reversible impaired immune functions in THP-1 cells. A & B, Treatment with 50 ng/mL ethidium bromide (EtBr) for 8 weeks. A, MtDNA levels. B, LPS-induced TNF-α & IL-8 release. C-E, Transfection with 30 nmol/L negative or TFAM siRNA for 8 days. C, TFAM protein relative to β-actin. D, MtDNA levels. E, LPS-induced TNF-α & IL-8 release. F-H, TFAM recovery 8 days after removal of TFAM siRNA. F, TFAM protein relative β-actin. G, MtDNA levels. H, LPS-induced TNF-α & IL-8 release. All experiments are presented as means ± SD of 3 to 4 independent biological replicates. *P < .05, **P < .01, & ***P < .001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28629747), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Western Blot: mtTFA Antibody (18G102B2E11) [NBP1-71648] - MtDNA depletion & impaired immune functions & subsequent recovery in THP-1 cells following transfection with TFAM siRNA. A-G, Transfection with TFAM siRNA. A, TFAM proteins levels relative to β-actin during titration of TFAM siRNA, showing optimal knockdown of TFAM protein after transfection of THP-1 cells with 30 nmol/L siRNA for 8 days. B, Cell viability. C, Cell proliferation. D, The levels of the MT-CO1 & SDHA proteins relative to β-actin. D, OCR for different aspects of mitochondrial respiration & respiratory profile. E, Phagocytosis of E coli. Recovery 8 days after removal of TFAM siRNA. F, Levels of the MT-CO1 & SDHA proteins relative to β-actin. G, Oxygen consumption for different aspects of mitochondrial respiration. H, Bacterial phagocytosis. All experiments were carried out on 3 to 4 independent biological replicates & are presented as means ± SD. **P < .01 & ***P < .001. Image collected & cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/28629747), licensed under a CC-BY license. Not internally tested by Novus Biologicals.



Publications

Xu W, Zhao L. An Enzyme-Linked Immunosorbent Assay for the Detection of Mitochondrial DNA-Protein Cross-Links from Mammalian Cells DNA (Basel) 2023-08-21 [PMID: 37601565] (Western Blot, Rat)

Dey S, Catchpole T, Takacs A, Csaky KG. et Al. Investigating the effects of 7-ketocholesterol on retinal pigment epithelium bioenergetics FASEB J 2023-05-30 [PMID: 37249566]

Zhang C, Shen S, Xu L et Al. LONP1 alleviates ageing-related renal fibrosis by maintaining mitochondrial homeostasis J Cell Mol Med 2024-09-11 [PMID: 39261902]

Xu R, Huang L, Liu J et al. Remodeling of Mitochondrial Metabolism by a Mitochondria-Targeted RNAi Nanoplatform for Effective Cancer Therapy Small (Weinheim an der Bergstrasse, Germany) 2023-11-02 [PMID: 37919865]

Vidali S, Feichtinger RG, Emberger M et al. Ageing is associated with a reduction in markers of mitochondrial energy metabolism in the human epidermis Experimental dermatology 2023-02-27 [PMID: 36851889] (Immunohistochemistry-Paraffin, Human)

Ilamathi H, Benhammouda S, Lounas A et al. Contact sites between endoplasmic reticulum sheets and mitochondria regulate mitochondrial DNA replication and segregation iScience 2023-06-01 [PMID: 37534187] (ICC/IF, Human)

Saal KA, Shaib AH, Mougios N et al. Heat denaturation enables multicolor X10-STED microscopy Scientific reports 2023-04-01 [PMID: 37005431] (ICC/IF, Human)

Xu W, Zhao L An Enzyme-Linked Immunosorbent Assay for the Detection of Mitochondrial DNA-Protein Cross-Links from Mammalian Cells DNA 2022-11-11 (Western Blot, Human)

Gui J, Qiao W, Hu C et al. Chemotherapy potentiates CD8+ T cell cytotoxicity through stimulating cancer cell-autonomous type I IFN induction via oxidized mtDNA sensing Research Square 2022-12-06 (Immunocytochemistry/ Immunofluorescence, Mouse)

Limagne E, Nuttin L, Thibaudin M et al. MEK inhibition overcomes chemoimmunotherapy resistance by inducing CXCL10 in cancer cells Cancer cell 2022-01-17 [PMID: 35051357]

SA Harper, JR Bassler, S Peramsetty, Y Yang, LM Roberts, D Drummer, RT Mankowski, C Leeuwenbur, K Ricart, RP Patel, MM Bamman, SD Anton, BC Jaeger, TW Buford Resveratrol and exercise combined to treat functional limitations in late life: A pilot randomized controlled trial Exp Gerontol, 2020-10-15;0(0):111111. 2020-10-15 [PMID: 33068691] (Simple Western, Human)

Patrick S, Gowda P, Lathoria K Et al. Diminished YAP1 affects mitochondrial dynamics in IDH1 mutant glioma Journal of cell science 2021-10-15 [PMID: 34651186]

More publications at http://www.novusbio.com/NBP1-71648

Procedures

Western Blot protocol for mtTFA Antibody (NBP1-71648)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted 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 three 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.

*Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

Immunohistochemistry-Paraffin protocol for mtTFA Antibody (NBP1-71648)

Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes.

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in wash buffer for 5 minutes.
3. Block each section with 100-400 ul blocking solution for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul biotinylated diluted secondary antibody. Incubate 30 minutes at room temperature.
7. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
8. Add 100-400 ul Streptavidin-HRP reagent to each section and incubate for 30 minutes at room temperature.
9. Wash sections three times in wash buffer for 5 minutes each.
10. Add 100-400 ul DAB substrate to each section and monitor staining closely.
11. As soon as the sections develop, immerse slides in deionized water.
12. Counterstain sections in hematoxylin.
13. Wash sections in deionized water two times for 5 minutes each.
14. Dehydrate sections.
15. Mount coverslips.

Immunocytochemistry/Immunofluorescence protocol for mtTFA Antibody (NBP1-71648)

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 1:25,0000 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.

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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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