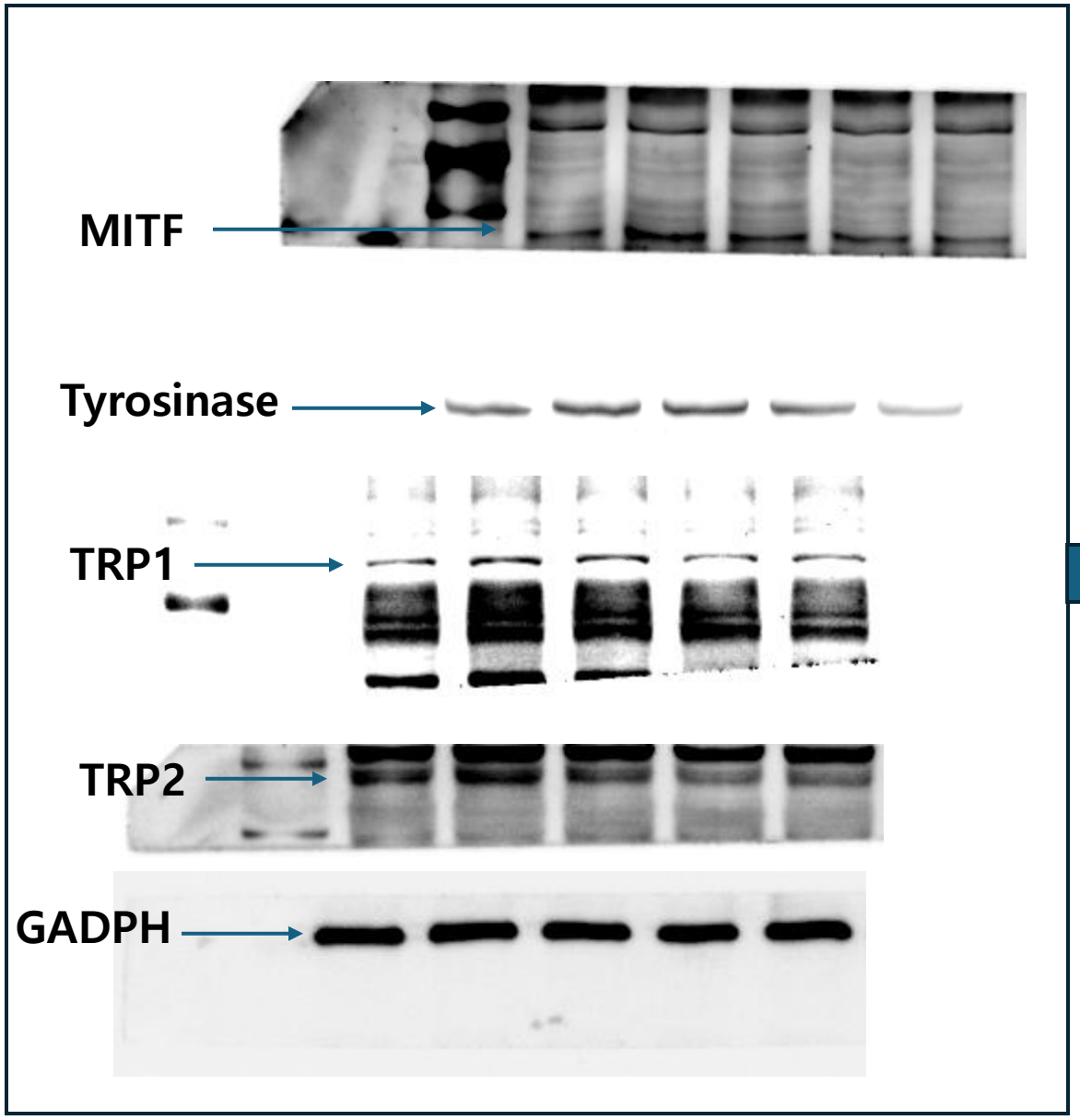
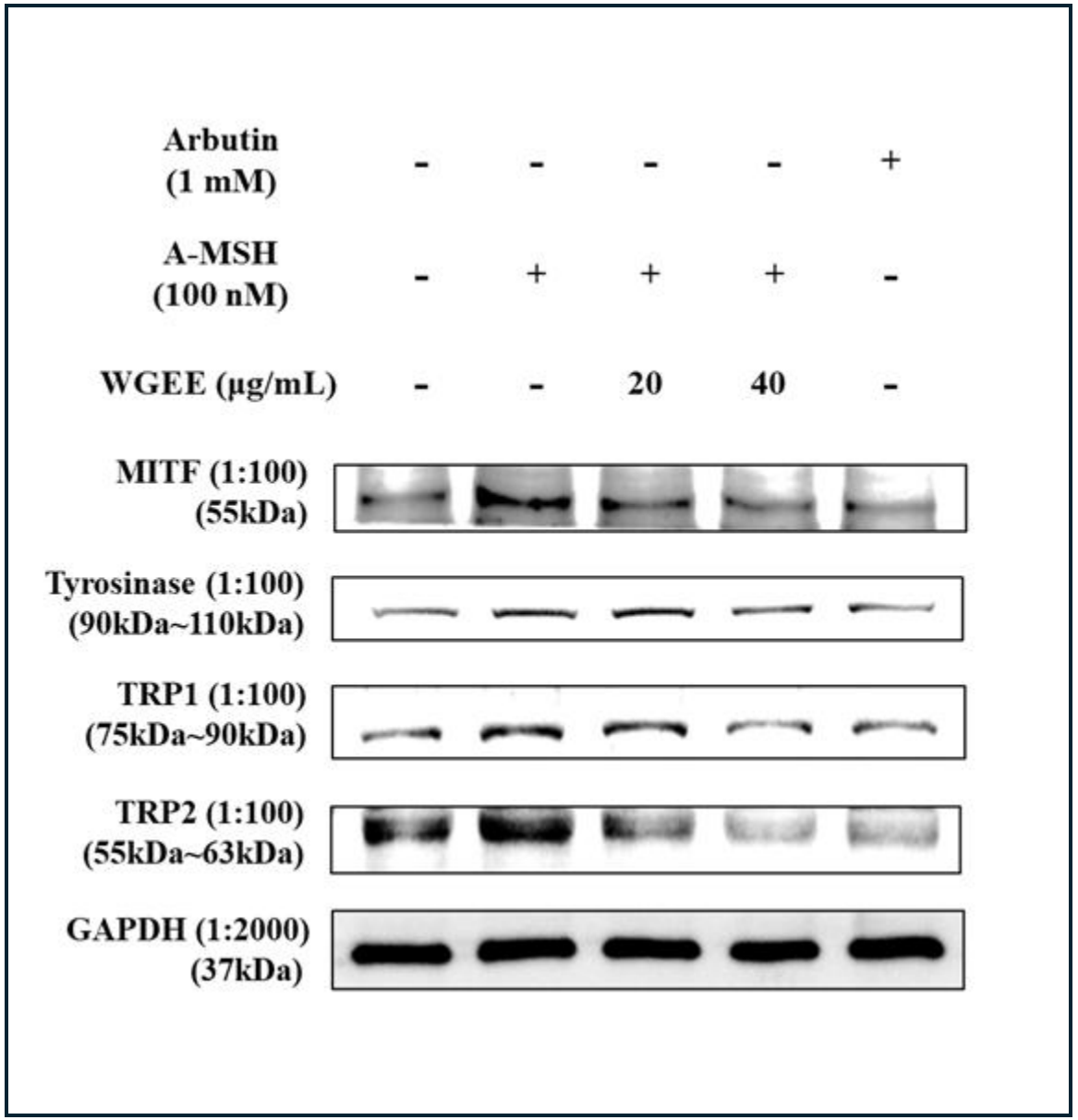
Supplementary data 2. Westen blot image

The full blot we shot



Placed in Figure 3A



MITF (63~85KDa)

SANTA CRUZ BIOTECHNOLOGY, INC.

MITF (D-9): sc-515925

BACKGROUND

MITF (microphthalmia-associated transcription factor) is a melanocytic nuclear protein that contains basic helix-loop-helix (HLH) and leucine zipper (LZ) domains. These protein motifs are frequently observed in other transcription factors and are particularly common to members of the Myc family. MITF can directly associate with DNA as a homodimer and is required for the development and differentiation of melanocytes. Its expression is upregulated by cAMP and cAMP-dependent pathways. MITF activates several different gene promoters by binding to their E-boxes. Tyrosinase, TRP1 and TRP2 are pigment synthesis genes activated by MITF. When MITF is phosphorylated on Ser73 (via the MAPK pathway), it associates with co-activators of the p300/CBP family and enhances transcription. MITF has several isoforms including MITF-M which is specifically expressed in melanocytes. In MITF-deficient mice there is a complete absence of melanocytes.

REFERENCES

1. Beckmann, H., et al. 1990. TFE3: a helix-loop-helix protein that activates transcription through the immunoglobulin enhancer μE3 motif. Genes Dev. 4: 167-179.
2. Fisher, D.E., et al. 1991. TFEB has DNA-binding and oligomerization properties of a unique helix-loop-helix/leucine-zipper family. Genes Dev. 5: 2342-2352.
3. Kerkhoff, E., et al. 1991. Sequence-specific DNA binding by Myc proteins. Proc. Natl. Acad. Sci. USA 88: 4323-4327.

CHROMOSOMAL LOCATION

Genetic locus: MITF (human) mapping to 3p14.1; Mitf (mouse) mapping to 6 D3.

SOURCE

MITF (D-9) is a mouse monoclonal antibody specific for an epitope mapping between amino acids 41-61 near the N-terminus of MITF of human origin.

PRODUCT

Each vial contains 200 µg IgG_1 lambda light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin. Also available as TransCruz reagent for Gel Supershift and ChIP applications, sc-515925 X, 200 µg/0.1 ml.

MITF (D-9) is available conjugated to agarose (sc-515925 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-515925 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-515925 PE), fluorescein (sc-515925 FITC), Alexa Fluor® 488 (sc-515925 AF488), Alexa Fluor® 546 (sc-515925 AF546), Alexa Fluor® 594 (sc-515925 AF594) or Alexa Fluor® 647 (sc-515925 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-515925 AF680) or Alexa Fluor® 790 (sc-515925 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

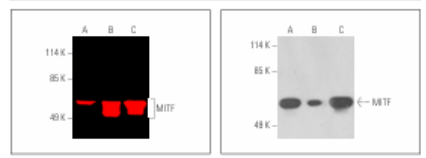
MITF (D-9) is recommended for detection of MITF of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for MITF siRNA (h): sc-35934, MITF siRNA (m): sc-35935, MITF shRNA Plasmid (h): sc-35934-SH, MITF shRNA Plasmid (m): sc-35935-SH, MITF shRNA (h) Lentiviral Particles: sc-35934-V and MITF shRNA (m) Lentiviral Particles: sc-35935-V.

MITF (D-9) X TransCruz antibody is recommended for Gel Supershift and ChIP applications.

Molecular Weight of MITF: 60 kDa.

DATA



MITF (D-9): sc-515925. Near-infrared western blot analysis of MITF expression in HeLa (**A**), SK-MEL-28 (**B**) and C32 (**C**) nuclear extracts. Blocked with UltraCruz® Blocking Reagent: sc-516214. Detection reagent used: m-IgGλ BP-CFL 790: sc-516195.

MITF (D-9) HRP: sc-515925 HRP. Direct western blot analysis of MITF expression in HeLa (**A**) and Jurkat (**B**) nuclear extracts and RPE-J whole cell lysate (**C**).

SELECT PRODUCT CITATIONS

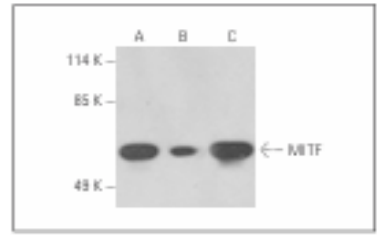
1. Huang, M., et al. 2018. A targeted quantitative proteomic approach assesses the reprogramming of small GTPases during melanoma metastasis. Cancer Res. 78: 5431-5445.
2. Ikarashi, N., et al. 2020. *Lactobacillus helveticus*-fermented milk whey suppresses melanin production by inhibiting tyrosinase through decreasing MITF expression. Nutrients 12: 2082.
3. Rachinger, N., et al. 2022. α-synuclein and its role in melanocytes. Cells 11: 2087.
4. Seefried, F., et al. 2022. Nuclear AREG affects a low-proliferative phenotype and contributes to drug resistance of melanoma. Int. J. Cancer 151: 2244-2264.
5. Lee, M.K., et al. 2022. Brassinin abundant in brassicaceae suppresses melanogenesis through dual mechanisms of tyrosinase inhibition. Foods 12: 121.

RESEARCH USE

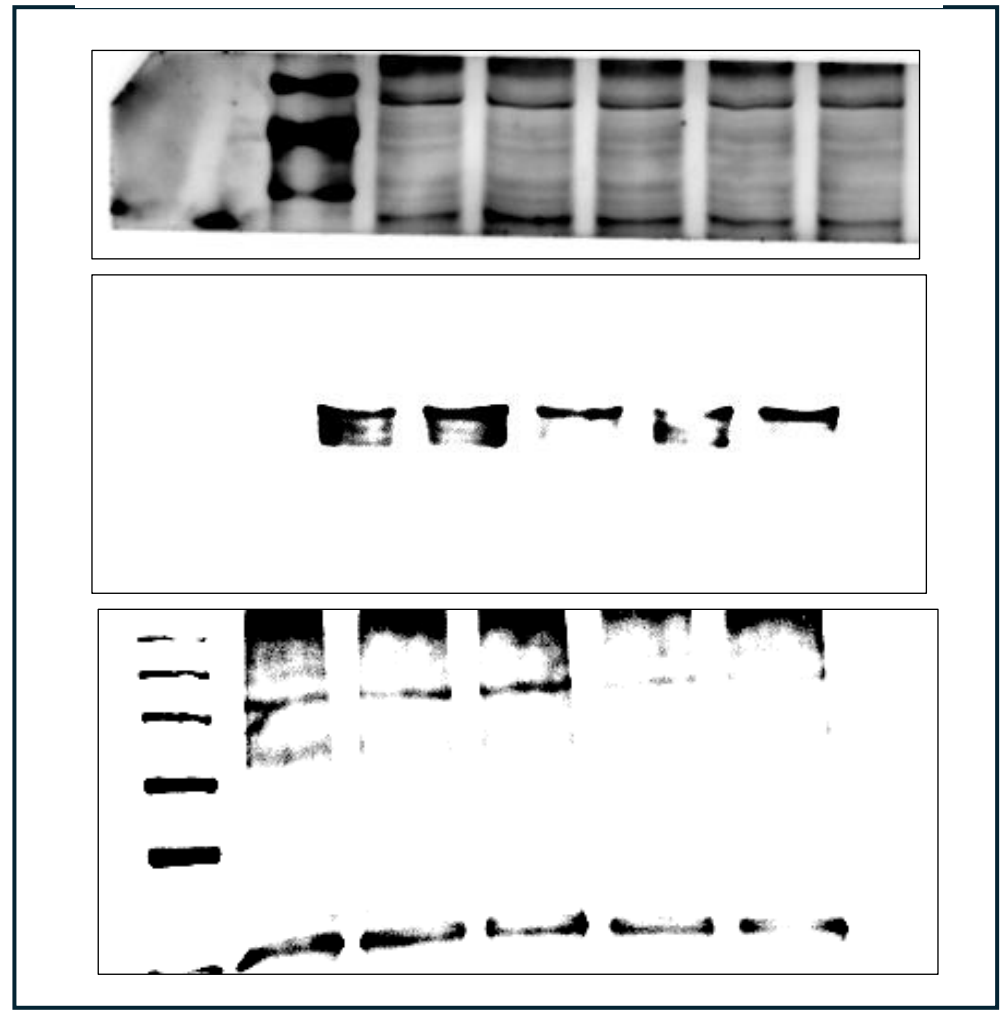
For research use only, not for use in diagnostic procedures.

DATA

MITF (D-9): sc-515925. Near-infrared western blot analysis of MITF expression in HeLa (**A**), SK-MEL-28 (**B**) and C32 (**C**) nuclear extracts. Blocked with UltraCruz® Blocking Reagent: sc-516214. Detection reagent used: m-IgGλ BP-CFL 790: sc-516195.



MITF (D-9) HRP: sc-515925 HRP. Direct western blot analysis of MITF expression in HeLa (**A**) and Jurkat (**B**) nuclear extracts and RPE-J whole cell lysate (**C**).



Tyrosinase (90kDa~110kDa)

SANTA CRUZ BIOTECHNOLOGY, INC.

Tyrosinase (T311): sc-20035

BACKGROUND

Tyrosinase (TYR), a type I membrane protein and copper-containing enzyme, is involved in the production of melanin, the primary pigment found in vertebrates. Melanin biogenesis requires the enzymatic activity of TYR, which catalyzes the critical and rate-limiting step of tyrosine hydroxylation in the biosynthesis of melanin. Defects effecting TYR activity result in various forms of albinism. The TYR-related proteins, TRP1 and TRP2, are also specifically expressed in melanocytes, and they likewise contribute to the synthesis of melanin within the melanosomes. The TRPs, including TYR, all share a similar transmembrane region, contain two metal-binding regions and a cysteine-rich epidermal growth factor motif, and are localized in the melanosomal membrane. These proteins, however, have distinct catalytic activity, and they individually contribute to the biosynthesis of melanin biopolymers. The TRPs are believed to exists as a multi-enzyme complex, as these proteins form aggregates together, and the expression of TRP1 also helps stabilize TYR in melanocytes.

CHROMOSOMAL LOCATION

Genetic locus: TYR (human) mapping to 11q14.3; Tyr (mouse) mapping to 7 D3.

SOURCE

Tyrosinase (T311) is a mouse monoclonal antibody raised against recombinant Tyrosinase of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2a} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Tyrosinase (T311) is available conjugated to agarose (sc-20035 AC), 500 µg/ 0.25 ml agarose in 1 ml, for IP; to HRP (sc-20035 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-20035 PE), fluorescein (sc-20035 FITC), Alexa Fluor® 488 (sc-20035 AF488), Alexa Fluor® 546 (sc-20035 AF546), Alexa Fluor® 594 (sc-20035 AF594) or Alexa Fluor® 647 (sc-20035 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-20035 AF680) or Alexa Fluor® 790 (sc-20035 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

APPLICATIONS

Tyrosinase (T311) is recommended for detection of Tyrosinase of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

Suitable for use as control antibody for Tyrosinase siRNA (h): sc-36766, Tyrosinase siRNA (m): sc-36767, Tyrosinase shRNA Plasmid (h): sc-36766-SH, Tyrosinase shRNA Plasmid (m): sc-36767-SH, Tyrosinase shRNA (h) Lentiviral Particles: sc-36766-V and Tyrosinase shRNA (m) Lentiviral Particles: sc-36767-V.

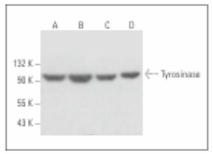
Molecular Weight of Tyrosinase: 60 kDa.

Molecular Weight of glycosylated Tyrosinase: 70-84 kDa.

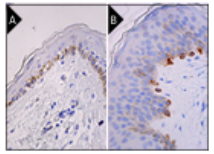
STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

DATA



Tyrosinase (T311): sc-20035. Western blot analysis of Tyrosinase expression in Hep G2 (**A**), Jurkat (**B**), A-431 (**C**) and A375 (**D**) whole cell lysates.



Tyrosinase (T311): sc-20035. Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of subset of basal epidermal cells (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of melanocytes (**B**).

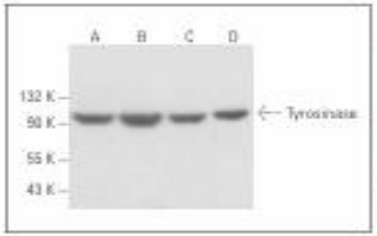
SELECT PRODUCT CITATIONS

1. Halaban, R., et al. 2000. Endoplasmic reticulum retention is a common defect associated with Tyrosinase-negative albinism. Proc. Natl. Acad. Sci. USA 97: 5889-5894.
2. Wang, D., et al. 2013. Optimization of the method for the culture of melanocyte precursors from hair follicles and their activation by 1,25-dihydroxyvitamin D_3. Exp. Ther. Med. 6: 967-972.
3. Bultema, J.J., et al. 2014. Myosin vc interacts with Rab32 and Rab38 proteins and works in the biogenesis and secretion of melanosomes. J. Biol. Chem. 289: 33513-33528.
4. Arts, N., et al. 2015. microRNA-155, induced by interleukin-1β, represses the expression of microphthalmia-associated transcription factor (MITF-M) in melanoma cells. PLoS ONE 10: e0122517.
5. Ambrosio, A.L., et al. 2016. TPC2 controls pigmentation by regulating melanosome pH and size. Proc. Natl. Acad. Sci. USA 113: 5622-5627.
6. Patwardhan, A., et al. 2017. Routing of the RAB6 secretory pathway towards the lysosome related organelle of melanocytes. Nat. Commun. 8: 15835.
7. Wu, Q., et al. 2018. Microphthalmia-associated transcription factor up-regulates acetylcholinesterase expression during melanogenesis of murine melanoma cells. J. Biol. Chem. 293: 14417-14428.
8. Teramae, A., et al. 2019. The molecular basis of chemical chaperone therapy for oculocutaneous albinism type 1A. J. Invest. Dermatol. 139: 1143-1149.

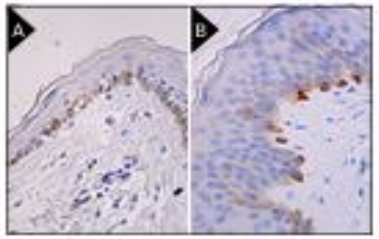
RESEARCH USE

For research use only, not for use in diagnostic procedures.

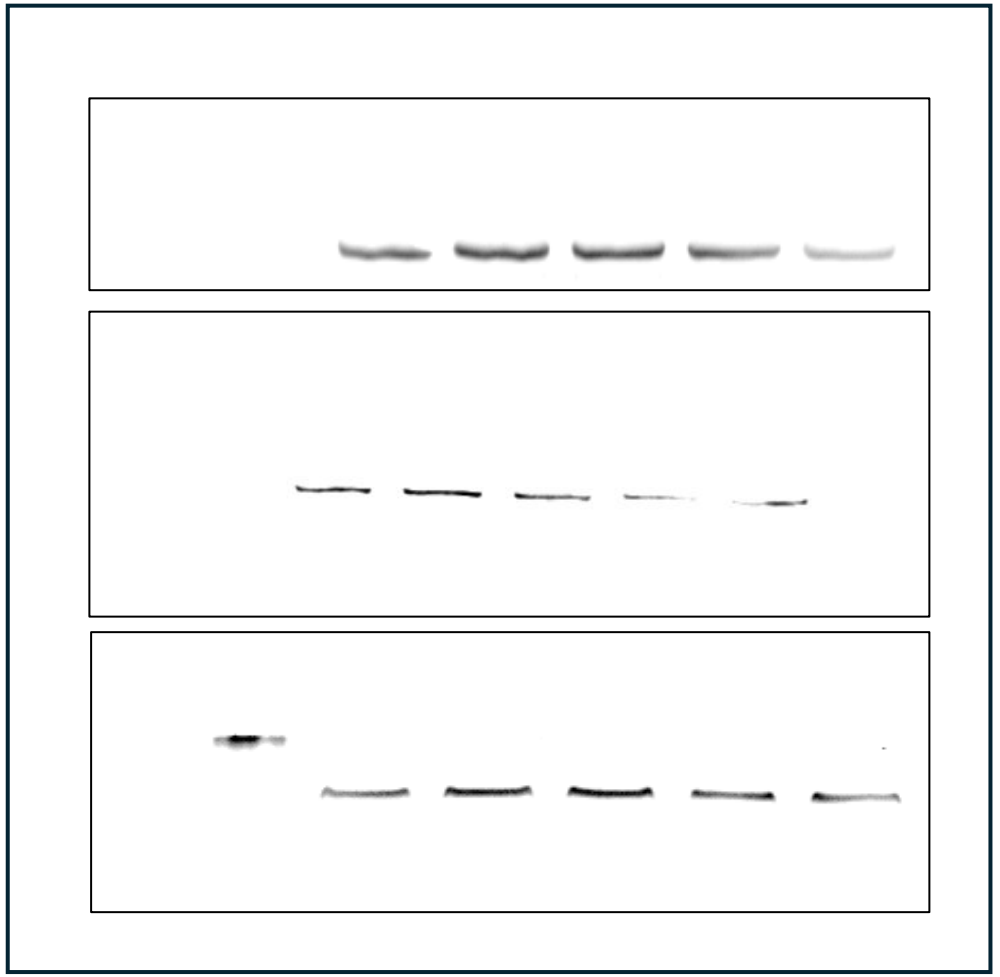
DATA



Tyrosinase (T311): sc-20035. Western blot analysis of Tyrosinase expression in Hep G2 (**A**), Jurkat (**B**), A-431 (**C**) and A375 (**D**) whole cell lysates.



Tyrosinase (T311): sc-20035. Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of subset of basal epidermal cells (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of melanocytes (**B**).



TRP1 (75~90kDa)

SANTA CRUZ BIOTECHNOLOGY, INC.

TRP1 (G-9): sc-166857

BACKGROUND

Tyrosinase (TYR), a type I membrane protein and copper-containing enzyme, is involved in the production of melanin, the primary pigment found in vertebrates. Melanin biogenesis requires the enzymatic activity of TYR, which catalyzes the critical and rate-limiting step of tyrosine hydroxylation in the biosynthesis of melanin. Defects effecting TYR activity result in various forms of albinism. The TYR-related proteins, TRP1 and TRP2, are also specifically expressed in melanocytes, and they likewise contribute to the synthesis of melanin within the melanosomes. The TRPs, including TYR, all share a similar transmembrane region, contain two metal-binding regions and a cysteine-rich epidermal growth factor motif, and are localized in the melanosomal membrane. These proteins, however, have distinct catalytic activity, and they individually contribute to the biosynthesis of melanin biopolymers. The TRPs are believed to exists as a multi-enzyme complex, as these proteins form aggregates together, and the expression of TRP1 also helps stabilize TYR in melanocytes.

CHROMOSOMAL LOCATION

Genetic locus: TYRP1 (human) mapping to 9p23; Tyrp1 (mouse) mapping to 4 C3.

SOURCE

TRP1 (G-9) is a mouse monoclonal antibody raised against amino acids 448-537 of TRP1 of human origin.

PRODUCT

Each vial contains 200 µg IgG_3 kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

APPLICATIONS

TRP1 (G-9) is recommended for detection of TRP1 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for TRP1 siRNA (h): sc-36745, TRP1 siRNA (m): sc-36744, TRP1 shRNA Plasmid (h): sc-36745-SH, TRP1 shRNA Plasmid (m): sc-36744-SH, TRP1 shRNA (h) Lentiviral Particles: sc-36745-V and TRP1 shRNA (m) Lentiviral Particles: sc-36744-V.

Molecular Weight of TRP1 depending on level of glycosylation: 70-90 kDa.

Positive Controls: B16-F0 cell lysate: sc-2298 or KNRK whole cell lysate: sc-2214.

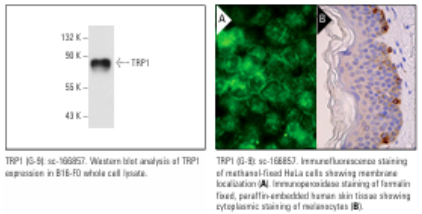
STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

DATA



TRP1 (G-9): sc-166857. Western blot analysis of TRP1 expression in B16-F0 whole cell lysate.

TRP1 (G-9): sc-166857. Immunofluorescence staining of methanol-fixed HeLa cells showing membrane localization (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of melanocytes (**B**).

SELECT PRODUCT CITATIONS

1. Poliakov, E., et al. 2014. Multiple A2E treatments lead to melanization of rod outer segment-challenged ARPE-19 cells. Mol. Vis. 20: 285-300.
2. Villareal, M.O., et al. 2017. Upregulation of mitf by phenolic compounds-rich *Cymbopogon schoenanthus* treatment promotes melanogenesis in B16 melanoma cells and human epidermal melanocytes. Biomed Res. Int. 2017: 8303671.
3. Müller-Dott, K., et al. 2019. Effect of sulfur mustard on melanogenesis *in vitro*. Toxicol. Lett. 319: 197-203.
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5. Effern, M., et al. 2020. Adoptive T cell therapy targeting different gene products reveals diverse and context-dependent immune evasion in melanoma. Immunity 53: 564-580.e9.
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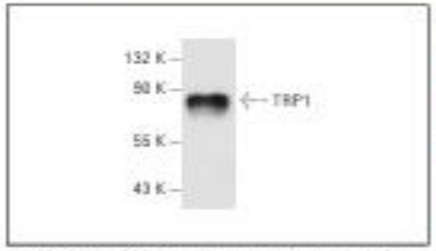
7. Shin, S., et al. 2021. Morin induces melanogenesis via activation of MAPK signaling pathways in B16F10 mouse melanoma cells. Molecules 26: 2150.
8. Guo, M.S., et al. 2021. A prepared platelet-rich plasma extract, namely self-growth colony, inhibits melanogenesis by down-regulating microphthalmia-associated transcription factor in skin melanocyte. J. Cosmet. Dermatol. 20: 3278-3288.
9. Isogawa, K., et al. 2021. Thioxothiazolidin derivative, 4-OST, inhibits melanogenesis by enhancing the specific recruitment of tyrosinase-containing vesicles to lysosome. J. Cell. Biochem. 122: 667-678.

PROTOCOLS

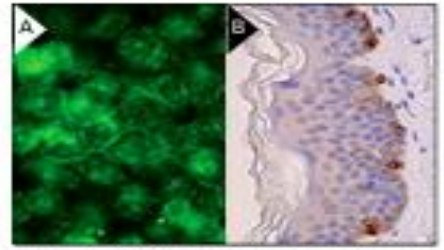
See our web site at www.scbt.com for detailed protocols and support products.

Santa Cruz Biotechnology, Inc. 1.800.457.3801 831.457.3800 fax 831.457.3801 **Europe** +00800 4573 8000 49 6221 4503 0 ***www.scbt.com***

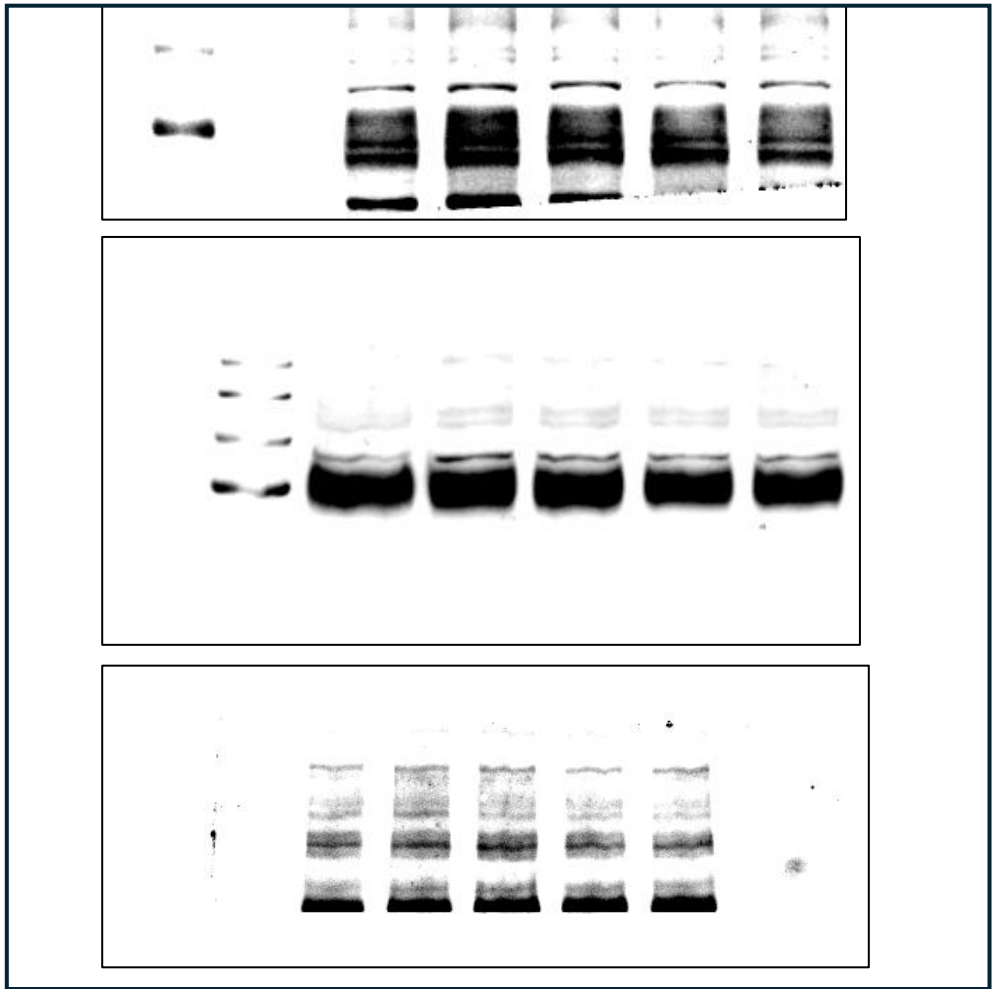
DATA



TRP1 (G-9): sc-166857. Western blot analysis of TRP1 expression in B16-F0 whole cell lysate.



TRP1 (G-9): sc-166857. Immunofluorescence staining of methanol-fixed HeLa cells showing membrane localization (**A**). Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic staining of melanocytes (**B**).



TRP2 (55kDa~63kDa)

SANTA CRUZ BIOTECHNOLOGY, INC.

TRP2 (C-9): sc-74439

BACKGROUND

Tyrosinase (TYR), a type I membrane protein and copper-containing enzyme, is involved in the production of melanin, the primary pigment found in vertebrates. Melanin biogenesis requires the enzymatic activity of TYR, which catalyzes the critical and rate-limiting step of tyrosine hydroxylation in the biosynthesis of melanin. Defects effecting TYR activity result in various forms of albinism. The TYR-related proteins, TRP1 and TRP2, are also specifically expressed in melanocytes, and they likewise contribute to the synthesis of melanin within the melanosomes. The TRPs, including TYR, all share a similar transmembrane region, contain two metal-binding regions and a cysteine-rich epidermal growth factor motif, and are localized in the melanosomal membrane. These proteins, however, have distinct catalytic activity, and they individually contribute to the biosynthesis of melanin biopolymers. The TRPs are believed to exists as a multi-enzyme complex, as these proteins form aggregates together, and the expression of TRP1 also helps stabilize TYR in melanocytes.

REFERENCES

1. Korner, A. and Pawelek, J. 1982. Mammalian tyrosinase catalyzes three reactions in the biosynthesis of melanin. Science 217: 1163-1165.
2. Shibahara, S., et al. 1986. Cloning and expression of cDNA encoding mouse tyrosinase. Nucleic Acids Res. 14: 2413-2427.
3. Hearing, V.J. and Jiménez, M. 1987. Mammalian tyrosinase—the critical regulatory control point in melanocyte pigmentation. Int. J. Biochem. 19: 1141-1147.

CHROMOSOMAL LOCATION

Genetic locus: DCT (human) mapping to 3q11.2; Dct (mouse) mapping to 14 E4.

SOURCE

TRP2 (C-9) is a mouse monoclonal antibody raised against amino acids 41-190 of TRP2 of human origin.

PRODUCT

Each vial contains 200 µg IgG_{2a} kappa light chain in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

TRP2 (C-9) is available conjugated to agarose (sc-74439 AC), 500 µg/0.25 ml agarose in 1 ml, for IP; to HRP (sc-74439 HRP), 200 µg/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-74439 PE), fluorescein (sc-74439 FITC), Alexa Fluor® 488 (sc-74439 AF488), Alexa Fluor® 546 (sc-74439 AF546), Alexa Fluor® 594 (sc-74439 AF594) or Alexa Fluor® 647 (sc-74439 AF647), 200 µg/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-74439 AF680) or Alexa Fluor® 790 (sc-74439 AF790), 200 µg/ml, for Near-Infrared (NIR) WB, IF and FCM.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

APPLICATIONS

TRP2 (C-9) is recommended for detection of TRP2 of mouse, rat and human origin by Western Blotting (starting dilution 1:100, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

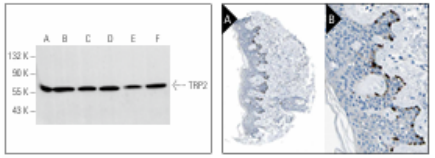
Suitable for use as control antibody for TRP2 siRNA (h): sc-41661, TRP2 siRNA (m): sc-41662, TRP2 shRNA Plasmid (h): sc-41661-SH, TRP2 shRNA Plasmid (m): sc-41662-SH, TRP2 shRNA (h) Lentiviral Particles: sc-41661-V and TRP2 shRNA (m) Lentiviral Particles: sc-41662-V.

Molecular Weight of TRP2 precursor: 59 kDa.

Molecular Weight of glycosylated TRP2: 75 kDa.

Positive Controls: K-562 whole cell lysate: sc-2203, HeLa whole cell lysate: sc-2200 or NCI-H460 whole cell lysate: sc-364235.

DATA



TRP2 (C-9): sc-74439. Western blot analysis of TRP2 expression in Y79 (**A**), K-562 (**B**), HeLa (**C**), NCI-H460 (**D**), MCF7 (**E**) and C8 (**F**) whole cell lysates.

TRP2 (C-9): sc-74439. Immunoperoxidase staining of formalin fixed, paraffin-embedded human skin tissue showing cytoplasmic and membrane staining of a subset of epidermal cells at low (**A**) and high (**B**) magnification. Kindly provided by The Swedish Human Protein Atlas (HPA) program.

SELECT PRODUCT CITATIONS

1. Bernard, D., et al. 2010. Processing of tumor antigen differentially impacts the development of helper and effector CD4+ T cell responses. Mol. Ther. 18: 1224-1232.
2. Jeon, S. and Kim, M.M. 2021. The down-regulation of melanogenesis via MITF and FOXO1 signaling pathways in SIRT1 knockout cells using CRISPR/Cas9 system. J. Biotechnol. 342: 114-127.
3. Fessé, P., et al. 2022. Human cutaneous interfollicular melanocytes differentiate temporarily under genotoxic stress. iScience 25: 105238.
4. Wagatsuma, T., et al. 2023. Pigmentation and TYRP1 expression are mediated by zinc through the early secretory pathway-resident ZNT proteins. Commun. Biol. 6: 403.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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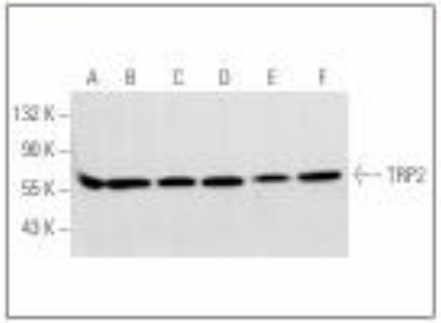
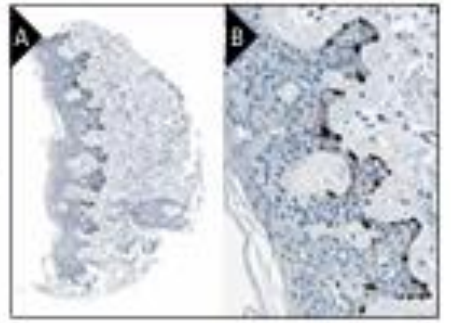
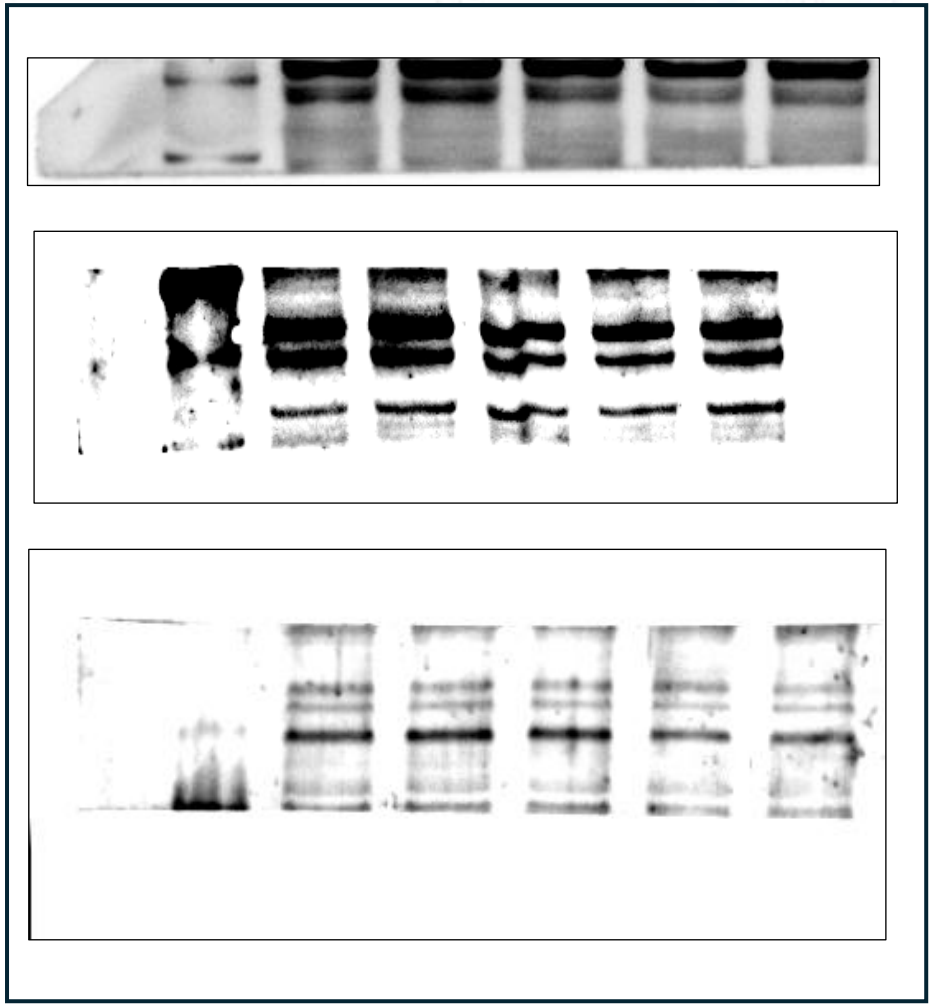
DATA







GADPH (37kDa)

Revision 8

Store at -20C

#2118

GAPDH (14C10) Rabbit mAb

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, W-S, IHC-P, IF-IC, FC-FP	H M R Mk B Pg	Endogenous	37	Rabbit	#P04406	2597

Product Usage Information

Application	Dilution
Western Blotting	1:1000
Simple Western™	1:10 - 1:50
Immunohistochemistry (Paraffin)	1:400 - 1:1600
Immunofluorescence (Immunocytochemistry)	1:50 - 1:200
Flow Cytometry (Fixed/Permeabilized)	1:100 - 1:400

Storage

Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.

For a carrier free (BSA and azide free) version of this product see product #85925.

Specificity/Sensitivity

GAPDH (14C10) Rabbit mAb detects endogenous levels of total GAPDH protein.

Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Lys260 of human GAPDH protein.

Background

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the phosphorylation of glyceraldehyde-3-phosphate during glycolysis. Though differentially expressed from tissue to tissue (1), GAPDH is thought to be a constitutively expressed housekeeping protein. For this reason, GAPDH mRNA and protein levels are often measured as controls in experiments quantifying specific changes in expression of other targets. Recent work has elucidated roles for GAPDH in apoptosis (2), gene expression (3), and nuclear transport (4). GAPDH may also play a role in neurodegenerative pathologies such as Huntington and Alzheimer's diseases (4,5).

Background References

1. Barber, R.D. et al. (2005) *Physiol. Genomics* 21, 389-95.
2. Hara, M.R. and Snyder, S.H. (2006) *Cell Mol. Neurobiol.* 26, 527-38.
3. Zheng, L. et al. (2003) *Cell* 114, 255-66.
4. Bae, B.I. et al. (2006) *Proc. Natl. Acad. Sci. USA* 103, 3405-9.
5. Wang, Q. et al. (2005) *FASEB J.* 19, 869-71.

Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

Western Blot Buffer

IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.

Applications Key

W: Western Blotting **W-S:** Simple Western™ **IHC-P:** Immunohistochemistry (Paraffin) **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

Cross-Reactivity Key

H: Human **M:** Mouse **R:** Rat **Mk:** Monkey **B:** Bovine **Pg:** Pig

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Limited Uses