

WRN | Validation File

TARGET WRN (Werner Syndrome protein)

CLONE NAME 195C

DESCRIPTION mouse monoclonal

ANTIGEN USED GST-WRN (1072aa-1432aa) recombinant protein

ISOTYPE lgG1

SPECIES REACTIVITY human and mouse

LOCALIZATION nuclea**r**

POSITIVE CONTROL Testicle

STORAGE BUFFER Tissue culture supernatant: 0.02% sodium azide

STORAGE Aliquot and store at 4C. Do not freeze











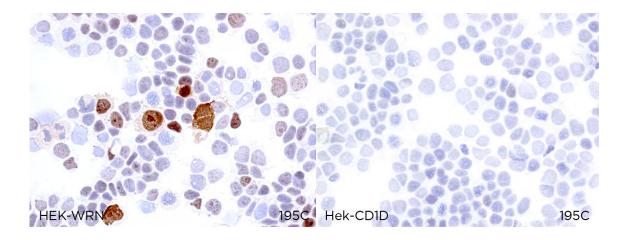
APPLICATIONS

| ICC | *Immunocytochemistry*

WRN195C is able to detect human WRN protein in immunocytochemistry

DILUTION neat supernatant

To confirm that WRN195C mAb recognizes human WRN protein, immunocytochemistry on frozen cytospin preparations of human WRN expressed in HEK293T was performed. Cytospin preparation of human CD1D protein was used as a negative control.





| WB | Western Blotting

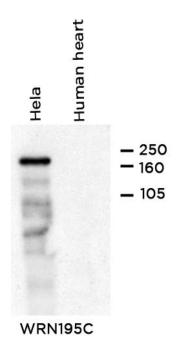
195C mAb is able to detect human and mouse WRN protein by WB.

DILUTION 1:3 supernatant

Predicted molecular weight: **162kDa** Observed molecular weight: **162kDa**

LANES

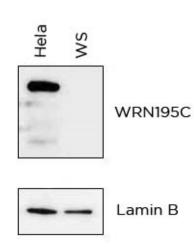
Lane 1 Hela cell line (200ug) (+) Lane 2 Human heart (200ug) (-)



LANES

Lane 1 Hela cell line (5 ul/lane, 50,000 cells/lane) (+) Lane 2 WS (AG11395, fibroblast of a patient with Werner Syndrome immortalized with SV40, 6 ul/lane, 60,000 cells/lane) (-)

Lamin B was used as a loading control



LANES

Lane 1 HCT116 cell line
Lane 2 HCT116 cell line
Lane 3 HCT116 cell line siRNA WRN
cells) (-)
Lane 4 HCT116 cell line siRNA WRN
cells) (-)

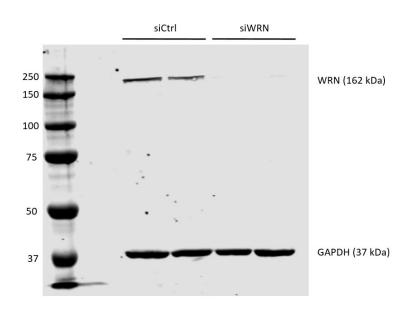
GAPDH was used as loading control

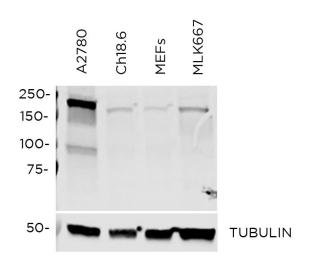
LANES

Lane 1 A2780 human ovarian cancer cell line (+)
Lane 2 Ch18.6 mouse lung cancer cell line (+)
Lane 3 MEFs (+)
Lane 4 MLK667 pancreatic cancer mouse cell line (+)

TUBULIN was used as loading control

Image kindly provided by Sonia Hernández





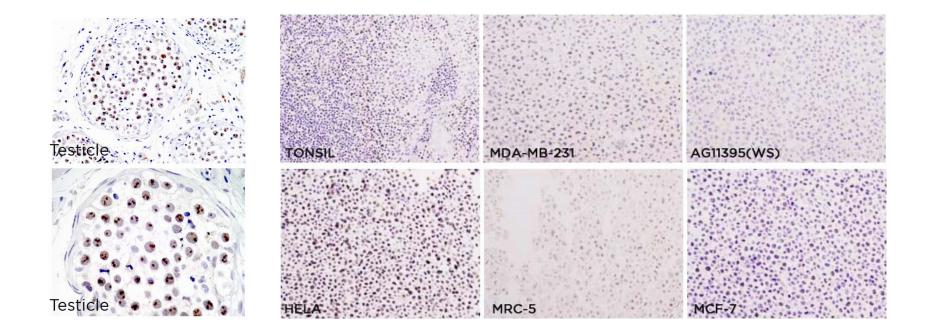


195C mAb can be used to detect WRN protein in human paraffin tissues

TISSUE SAMPLE Human testicle, human tonsil, MDA-MB-231 (breast adenocarcinoma cell line), AG11395 WS (fibroblast of a patient with Werner Syndrome immortalized with SV40, negative control), HeLa cell line, MRC-5 (lung fibroblast cell line) and MCF-7 (breast carcinoma cell line).

DILUTION 1:10 supernatant **ANT. RETRIEVAL** Citrate+PK

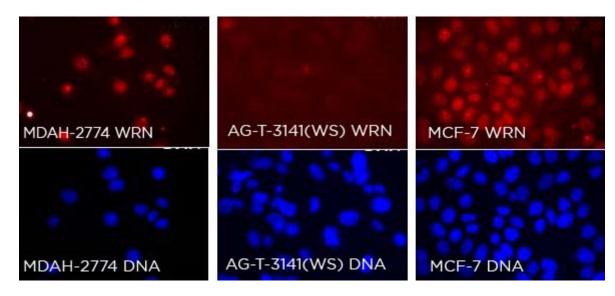
DETECTION SYSTEM Novolink kit (BondMax Leica)





195C mAb can be used to detect WRN protein by immunofluorescence

SAMPLE MDAH-2774 (Ovarian adenocarcinoma), AG-T-3141 (WS) (fibroblast WS patient, negative control) and MCF-7 (breast adenocarcinoma). **DILUTION** neat supernatant

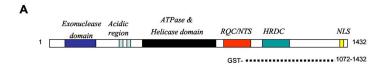


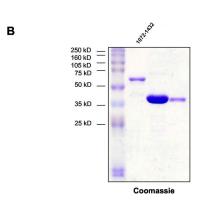
- | IHC-F | *Immunohistochemistry (frozen)* Not tested
- FC | Flow Cytometry Not tested
- | IP | *Immunoprecipitation* Not Tested

SOLD BY Abcam, Active Motif and Sigma

ADDITIONAL INFORMATION

- A. Functional domains of WRN and recombinant WRN fragment (1072-1432) corresponding to the antigen used for antibody production.
- B. WRN fragment was purified and analyzed by SDS-PAGE and visualized by Coomassie Blue staining.





This image has been obtained by the article: Von Kobbe C, Thomä NH, Czyzewski BK, Pavletich NP, Bohr VA. Werner syndrome protein contains three structure-specific DNA binding domains. J Biol Chem. 2003 Dec 26;278(52):52997-3006.

REFERENCES

Amalnath SD, Sargolzaeiaval F, Oshima J and Baskar D. Uncommon cause of cirrhosis-A case of Werner syndrome with a novel WRN mutation. Indian J Gastroenterol. 2017 Jul;36(4):323-325.

Kehrli K, Phelps M, Lazarchuk P, Chen E, Monnat R Jr and Sidorova JM. Class I Histone Deacetylase HDAC1 and WRN RECQ Helicase Contribute Additively to Protect Replication Forks upon Hydroxyurea-induced Arrest. J Biol Chem. 2016 Nov 18;291(47):24487-24503.

Welcsh P, Kehrli K, Lazarchuk P, Ladiges W, Sidorova J. Application of the microfluidic-assisted replication track analysis to measure DNA repair in human and mouse cells. Methods. 2016 Oct 1;108:99-110.

Li Z, Zhu Y, Zhai Y, R Castroagudin M, Bao Y, White TE, Glavy JS.Werner complex deficiency in cells disrupts the Nuclear Pore Complex and the distribution of lamin B1. Biochimica et Biophysica Acta on 1 December 2013.

Glukhova VA, Tomazela DM, Findlay GD, Monnat RJ Jr, MacCoss MJ.Rapid assessment of RNAi-mediated protein depletion by selected reaction monitoring mass spectrometry. Journal of Proteome Research on 5 July 2013.

Saha B, Lessel D, Nampoothiri S, Rao AS, Hisama FM, Peter D, Bennett C, Nürnberg G, Nürnberg P, Martin GM, Kubisch C, Oshima J. Ethnic-Specific WRN Mutations in South Asian Werner Syndrome Patients: Potential Founder Effect in Patients with Indian or Pakistani Ancestry. Molecular Genetics Genomic Medicine 1 May 2013.

Katrin Friedrich, Lin Lee, Dru F Leistritz, Gudrun Nürnberg, Bidisha Saha, Fuki M Hisama, Daniel K Eyman, Davor Lessel, Peter Nürnberg, Chumei Li, María J Garcia-F-Villalta, Carolien M Kets, Joerg Schmidtke, Vítor Tedim Cruz, Peter C Van den Akker, Joseph Boak, Dincy Peter, Goli Compoginis, Kivanc Cefle, Sukru Ozturk, Norberto López, Theda Wessel, Martin Poot, P F Ippel, Birgit Groff-Kellermann, Holger Hoehn, George M Martin, Christian Kubisch, Junko Oshima. WRN mutations in Werner syndrome patients: genomic rearrangements, unusual intronic mutations and ethnic-specific alterations. In Human Genetics. 1 July 2010.

Patricia L Opresko, José Palacios Calvo, Cayetano von Kobbe. Role for the Werner syndrome protein in the promotion of tumor cell growth. Mechanisms of Ageing and Development. 13 July 2007.