abcam

Product datasheet

Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade ab220788



4 References 14 Images

Overview

Product name Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade

Description Rabbit monoclonal [EPR21954] to TATA binding protein TBP - ChIP Grade

Host species Rabbit

Tested applications

Suitable for: ChIP-sequencing, Flow Cyt (Intra), ChIP, ChIC/CUT&RUN-seq, ICC/IF, WB, IHC-P,

IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, HEK-293, HCT 116, MCF7, NIH/3T3 and C2C12 whole cell lysates; mouse and rat

testis lysates. IP: HeLa whole cell lysate. IHC-P: Human testis and bladder cancer tissue; Mouse and rat testis tissues. ICC/IF: HeLa and HCT 116 cells. Flow Cyt (intra): HeLa cells. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells.

ChlC/CUT&RUN-Seq: HeLa cells.

General notesThis product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**[®] **patents**.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

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Purity Protein A purified

Clonality Monoclonal
Clone number EPR21954

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab220788 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
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
Flow Cyt (Intra)		1/500.
ChIP		Use 5 µg for 25 µg of chromatin.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 3µg
ICC/IF		1/500.
WB		1/1000. Detects a band of approximately 45, 35 kDa (predicted molecular weight: 38 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/30.

Target

Function

General transcription factor that functions at the core of the DNA-binding multiprotein factor TFIID. Binding of TFIID to the TATA box is the initial transcriptional step of the pre-initiation complex (PIC), playing a role in the activation of eukaryotic genes transcribed by RNA polymerase II. Component of the transcription factor SL1/TIF-IB complex, which is involved in the assembly of the PIC (preinitiation complex) during RNA polymerase I-dependent transcription. The rate of PIC formation probably is primarily dependent on the rate of association of SL1 with the rDNA promoter. SL1 is involved in stabilization of nucleolar transcription factor 1/UBTF on rDNA.

Tissue specificity

Widely expressed, with levels highest in the testis and ovary.

Involvement in disease

Defects in TBP are the cause of spinocerebellar ataxia type 17 (SCA17) [MIM:607136]. Spinocerebellar ataxia is a clinically and genetically heterogeneous group of cerebellar disorders. Patients show progressive incoordination of gait and often poor coordination of hands, speech and eye movements, due to degeneration of the cerebellum with variable involvement of the brainstem and spinal cord. SCA17 is an autosomal dominant cerebellar ataxia (ADCA) characterized by widespread cerebral and cerebellar atrophy, dementia and extrapyramidal signs. The molecular defect in SCA17 is the expansion of a CAG repeat in the coding region of TBP. Longer expansions result in earlier onset and more severe clinical manifestations of the

disease.

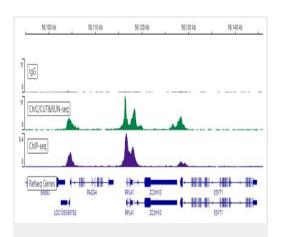
Sequence similarities

Belongs to the TBP family.

Cellular localization

Nucleus.

Images



ChIC/CUT&RUN sequencing - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 3×10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and $3\mu g$ of ab220788 [EPR21954]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control <u>ab172730</u> is also shown.

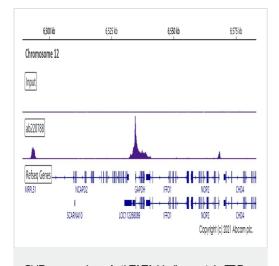
The ChIP data was conducted on chromatin prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 μg of chromatin and 4 μg of ab220788 with ChIP-Kit Transcription Factors ChIP-Seq (ab270813). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded <u>here</u>.

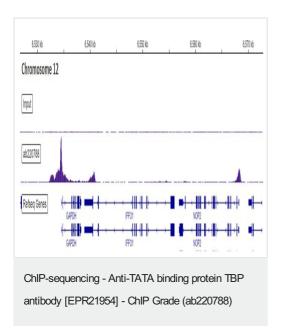
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 μg of chromatin and 4 μg of ab220788. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. ChIP-Seq validation performed with ChIP-Kit Transcription Factors ChIP-Seq (ab270813).

Additional screenshots of mapped reads can be downloaded **here**.

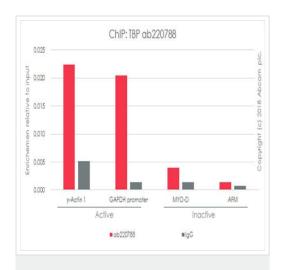


ChIP-sequencing - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)



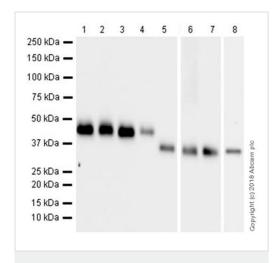
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 μ g of chromatin and 4 μ g of Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)

Chromatin was prepared from HeLa (human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. The ChIP was performed with 25 μ g of chromatin, 5 μ g of ab220788 (red), and 20 μ l of Protein A/G sepharose beads. 5 μ g of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (SYBR green approach).Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)

All lanes : Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788) at 1/1000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: HEK-293 (human epithelial cell line from embryonic kidney) whole cell lysate

Lane 3 : HCT 116 (human colorectal carcinoma cell line) whole cell lysate

Lane 4 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate

Lane 5: C2C12 (mouse myoblast cell line) whole cell lysate

Lane 6 : Mouse testis lysate

Lane 7 : Rat testis lysate

Lane 8 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell

lysate

Lysates/proteins at 20 µg per lane.

Secondary

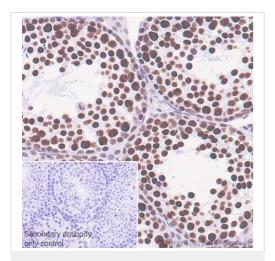
All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 38 kDa **Observed band size:** 35,45 kDa

Exposure times : Lanes 1-7: 15 seconds, Lane 8: 3 minutes.

Blocking and dilution buffer: 5% NFDM/TBST.

Human TBP migrates with an approximate molecular mass of 45 kDa on SDS-PAGE (PMID:1458534; PMID: 1907890) the molecular mass observed is lower in mouse than in human samples due to a shorter sequence.

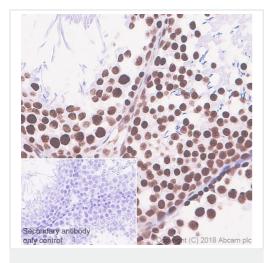


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TATA binding protein
TBP antibody [EPR21954] - ChIP Grade (ab220788)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling TATA binding protein TBP with ab220788 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in mouse testis (PMID: 17570761; PMID: 11861477) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

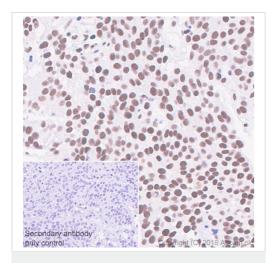


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TATA binding protein
TBP antibody [EPR21954] - ChIP Grade (ab220788)

Immunohistochemical analysis of paraffin-embedded rat testis tissue labeling TATA binding protein TBP with ab220788 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in rat testis (PMID: 17570761; PMID: 11861477) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

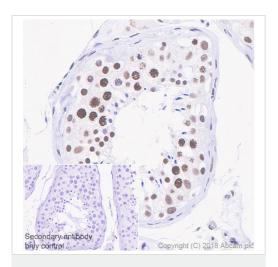


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TATA binding protein
TBP antibody [EPR21954] - ChIP Grade (ab220788)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling TATA binding protein TBP with ab220788 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in human bladder cancer (PMID: 17570761; PMID: 11861477) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.

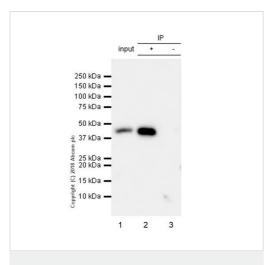


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TATA binding protein
TBP antibody [EPR21954] - ChIP Grade (ab220788)

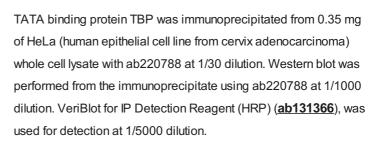
Immunohistochemical analysis of paraffin-embedded human testis tissue labeling TATA binding protein TBP with ab220788 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ready to use. Nuclear staining in human testis (PMID: 17570761; PMID: 11861477) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)



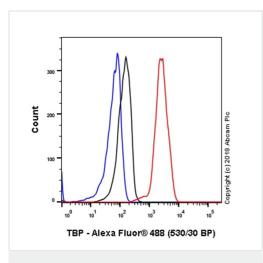
Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: ab220788 IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab220788 in HeLa whole cell lysate.

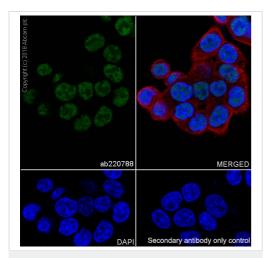
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 30 seconds.



Flow Cytometry (Intracellular) - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)

Intracellular flow cytometric analysis of 4% paraformal dehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling TATA binding protein TBP with ab 220788 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (ab 172730) (black) and an unlabeled control (cells incubated with secondary antibody only) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) (ab 150077), at 1/2000 dilution was used as the secondary antibody.

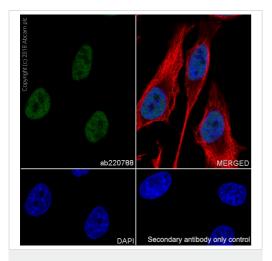


Immunocytochemistry/ Immunofluorescence - Anti-TATA binding protein TBP antibody [EPR21954] -ChIP Grade (ab220788)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (human colorectal carcinoma cell line) cells labeling TATA binding protein TBP with ab220788 at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HCT 116 cells. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.

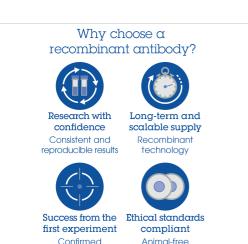


Immunocytochemistry/ Immunofluorescence - Anti-TATA binding protein TBP antibody [EPR21954] - ChIP Grade (ab220788)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling TATA binding protein TBP with ab220788 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining in HeLa cells. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor $^{(8)}$ 488) (ab150077) secondary antibody at 1/1000 dilution.



Confirmed Animal-free specificity production

Anti-TATA binding protein TBP antibody [EPR21954]

- ChIP Grade (ab220788)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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