


Anti-Oct4 antibody [EPR17929] - ChIP Grade ab181557

 RabMab

★★★★★ [20 Abreviews](#) [106 References](#) [13 图像](#)

概述

产品名称	Anti-Oct4抗体[EPR17929] - ChIP Grade
描述	兔单克隆抗体[EPR17929] to Oct4 - ChIP Grade
宿主	Rabbit
特异性	<p>Oct4 is highly expressed in undifferentiated embryonic stem cells and cancer stem cell-like cells (PMID: 26013162, 21826175).</p> <p>This antibody can't detect the target band in undifferentiated cancer cell lines with low expression level of Oct4, such as HeLa, HEK-293, MDA-MB-231, HepG2, Huh7, HCT-116 and PANC-1 (PMID: 21975933, 29789579, 25625591, 26059097, 23928699, 27344963, 25837691, 29254202, 28854261, 27996162), even at the dilution of 1:200.</p>
经测试应用	适用于: WB, ICC/IF, IP, IHC-P, ChIP-seq, ChIP, Flow Cyt (Intra)
种属反应性	<p>与反应: Mouse, Human</p> <p>预测可用于: Rat, Sheep, Horse, Cow, Pig, Common marmoset </p>
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: NCCIT, F9, and NTERA-2 cl.D1 whole cell lysates. IHC-P: Human seminoma and dysgerminoma of ovary tissues. ICC/IF: NCCIT cells. IP: NCCIT whole cell extract. ChIP: Chromatin prepared from F9 cells. ChIP-seq: NCCIT cells. Flow cyto(intra): NCCIT cells.
常规说明	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMab[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab[®] patents.</p>

性能

形式	Liquid
存放说明	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.

存储溶液	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR17929
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab181557于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

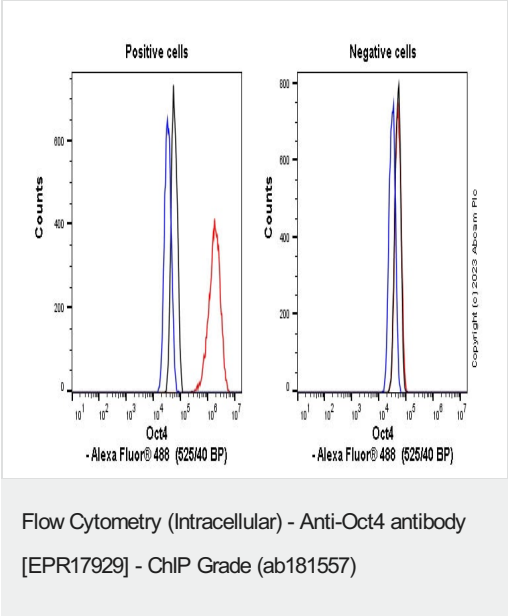
应用	Ab评论	说明
WB	★★★★★ (3)	1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 39 kDa).
ICC/IF	★★★★★ (8)	1/250.
IP		1/50.
IHC-P	★★★★★ (1)	1/1000. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse as there is no suitable positive tissue in-house.
ChIP-sequencing		Use at an assay dependent concentration.
ChIP	★★★★★ (1)	Use 5 µg for 25 µg of chromatin.
Flow Cyt (Intra)		Use at an assay dependent concentration. Purified format.

靶标

功能	Transcription factor that binds to the octamer motif (5'-ATTGTCAT-3'). Forms a trimeric complex with SOX2 on DNA and controls the expression of a number of genes involved in embryonic development such as YES1, FGF4, UTF1 and ZFP206. Critical for early embryogenesis and for embryonic stem cell pluripotency.
组织特异性	Expressed in developing brain. Highest levels found in specific cell layers of the cortex, the olfactory bulb, the hippocampus and the cerebellum. Low levels of expression in adult tissues.
序列相似性	Belongs to the POU transcription factor family. Class-5 subfamily. Contains 1 homeobox DNA-binding domain. Contains 1 POU-specific domain.
发展阶段	Highly expressed in undifferentiated embryonic stem cells and expression decreases gradually after embryoid body (EB) formation.
结构域	The POU-specific domain mediates interaction with PKM2.

翻译后修饰	<p>Sumoylation enhances the protein stability, DNA binding and transactivation activity. Sumoylation is required for enhanced YES1 expression.</p> <p>Ubiquitinated; undergoes 'Lys-63'-linked polyubiquitination by WWP2 leading to proteasomal degradation.</p>
细胞定位	Nucleus. Expressed in a diffuse and slightly punctuate pattern.

图片



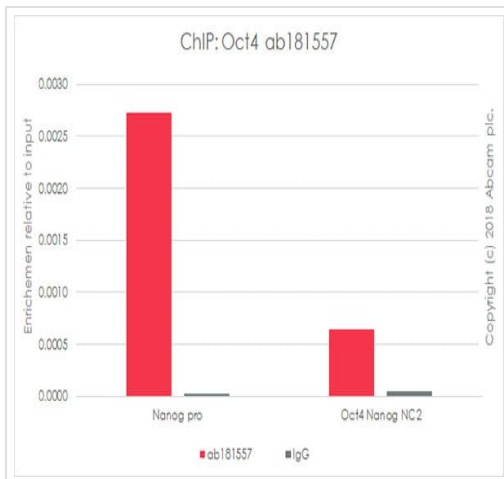
Flow cytometry overlay histogram showing left NCCIT positive cells and right negative HeLa stained with ab181557 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab181557) (1x 10⁶ in 100µl at 0.2µg/ml (1/10300)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

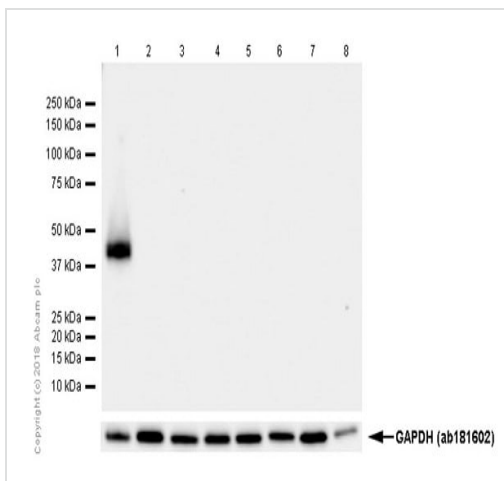
Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in NCCIT Fixed with 80% methanol (5 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



ChIP - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)



Western blot - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Chromatin was prepared from F9 (Mouse embryo testicular cancer cell line) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab181557 (red, and 20µl of Anti rabbit IgG sepharose beads. 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

“pro” stands for promoter region, while “NC2” stands for negative control which is negative loci at the promoter region.

All lanes : Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557) at 1/2000 dilution

Lane 1 : NCCIT (Human pluripotent embryonic carcinoma epithelial cell) whole cell lysates

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

Lane 3 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysates

Lane 4 : MDA-MB-231 (Human breast adenocarcinoma epithelial cell) whole cell lysates

Lane 5 : HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates

Lane 6 : Huh7 (Human hepatocellular carcinoma epithelial cell) whole cell lysates

Lane 7 : HCT 116 (Human colorectal carcinoma epithelial cell) whole cell lysates

Lane 8 : PANC-1 (Human pancreatic epithelioid carcinoma epithelial cell) whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

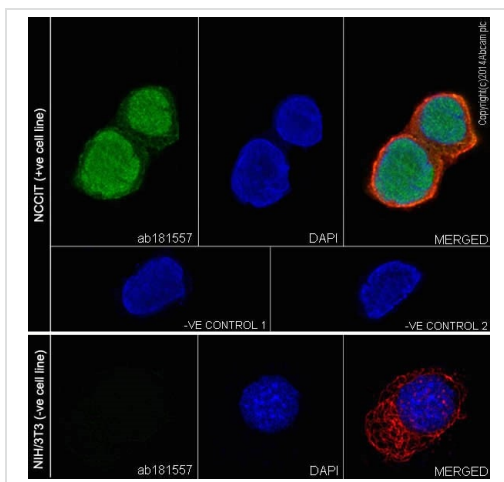
Predicted band size: 39 kDa

Observed band size: 45 kDa

Exposure time: 3 seconds

Blocking and diluting buffer: 5% NFDM/TBST

Oct4 is highly expressed in undifferentiated embryonic stem cells and cancer stem cell-like cells (PMID: 26013162, 21826175). ab181557 can't detect the target band in undifferentiated cancer cell lines with low expression level of Oct4, such as HeLa, HEK-293, MDA-MB-231, HepG2, Huh7, HCT-116 and PANC-1 (PMID: 21975933, 29789579, 25625591, 26059097, 23928699, 27344963, 25837691, 29254202, 28854261, 27996162), even at the dilution of 1:200.



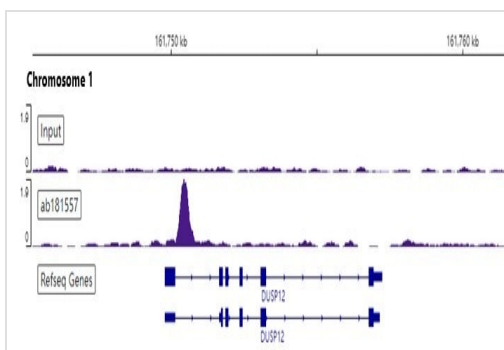
Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NCCIT (Human pluripotent embryonic carcinoma) cells (positive cell line) or NIH/3T3 (Mouse embryo fibroblast) cells (negative cell line) labeling Oct4 with ab181557 at 1/250 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Confocal image showing nuclear and weakly cytoplasmic staining on NCCIT cell line. Negative expression in NIH/3T3 cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: ab181557 at 1/250 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.

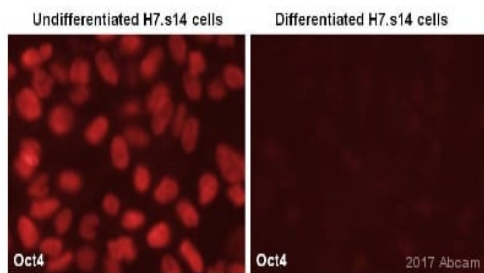
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



ChIP-sequencing - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Chromatin was prepared from NCCIT (Human pluripotent embryonic carcinoma cell line) cells. ChIP was performed with 10^7 NCCIT cells and 8 μ g of ab181557 [EPR17929]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

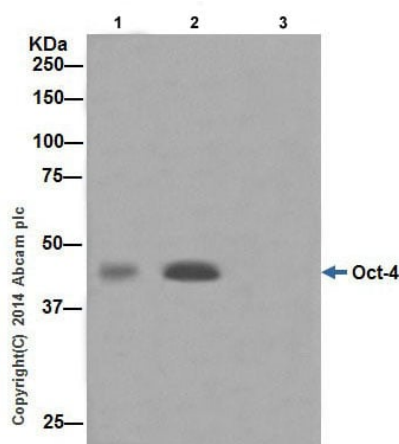
Additional screenshots of mapped reads can be downloaded [here](#).



Immunocytochemistry/ Immunofluorescence - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

This image is courtesy of an Abreview submitted by Joanne Lacey.

ab181557 staining Oct4 in Human embryonic stem cells by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with formaldehyde , permeabilized with 0.1% Triton in PBS for 1 hour and blocked with 10% Serum for 1 hour at 25°C. Samples were incubated with primary antibody (1/200 in PBS with 0.1% Tween20) for 16 hours at 4°C. A monoclonal Goat Anti-rabbit Alexa Fluor® 594 was used as the secondary antibody at 1/200 dilution.

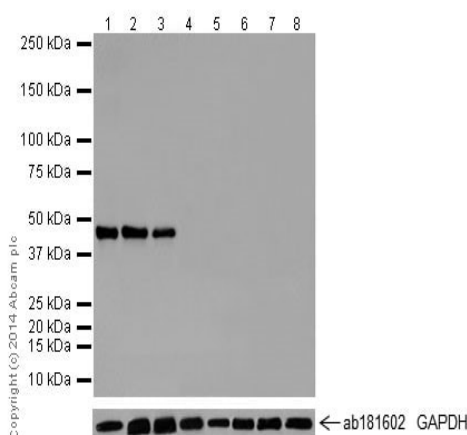


Immunoprecipitation - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Oct4 was immunoprecipitated from 1mg of NCCIT (Human pluripotent embryonic carcinoma) whole cell extract with ab181557 at 1/50 dilution. Western blot was performed from the immunoprecipitate using ab181557 at 1/10000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG, was used as secondary antibody at 1/1500 dilution.

Lane 1: NCCIT whole cell extract 10 µg (Input). Lane 2: ab181557 IP in NCCIT whole cell extract. Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab181557 in NCCIT whole cell extract. Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes



Western blot - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

All lanes : Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557) at 1/1000 dilution

Lane 1 : NCCIT (Human pluripotent embryonic carcinoma) whole cell lysate

Lane 2 : F9 (Mouse embryo testicular cancer cell line) whole cell lysate

Lane 3 : NTERA-2 cl.D1 (Human malignant pluripotent embryonic carcinoma) whole cell lysate

Lane 4 : Mouse testis lysate

Lane 5 : Human hippocampus lysate

Lane 6 : Human cerebellum lysate

Lane 7 : Human testis lysate

Lane 8 : NIH/3T3 (Mouse embryo fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

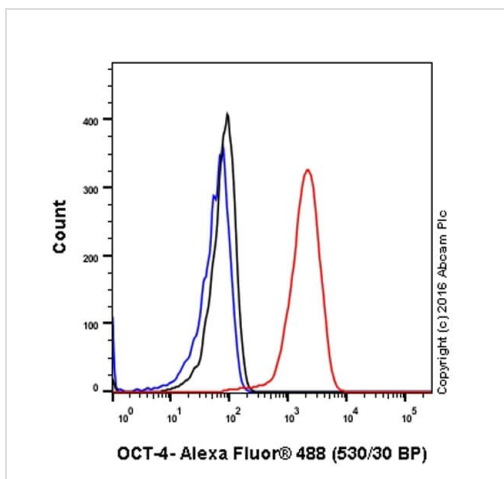
Predicted band size: 39 kDa

Observed band size: 45 kDa

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

Low levels of expression in adult tissues

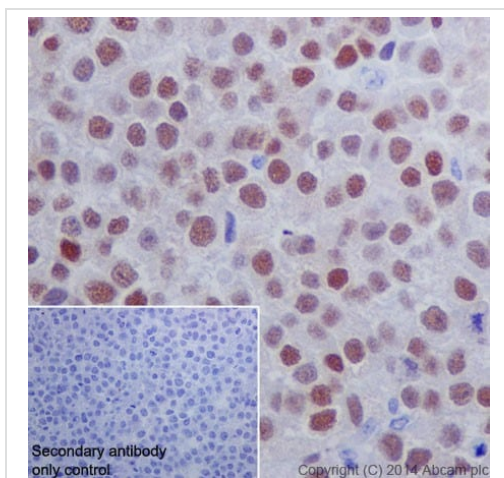


Flow Cytometry (Intracellular) - Anti-Oct4 antibody
[EPR17929] - ChIP Grade (ab181557)

ab181557 staining OCT-4 in the human cell line NCCIT (human pluripotent embryonal carcinoma) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/70. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody (Red).

Isotype control: Rabbit IgG monoclonal [EPR25A] **ab172730** (Black).

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue).

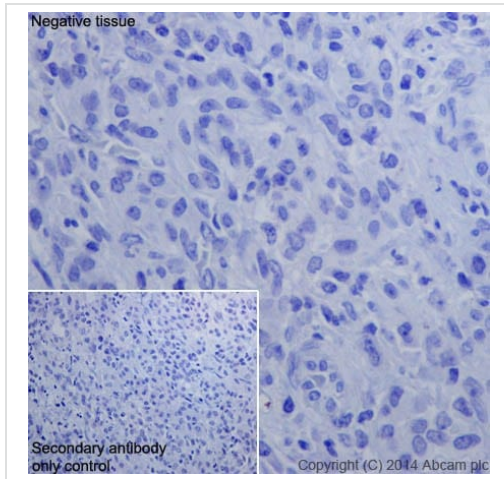


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody
[EPR17929] - ChIP Grade (ab181557)

Immunohistochemical analysis of paraffin-embedded Human dysgerminoma of ovary tissue labeling Oct4 with ab181557 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nuclear and weak cytoplasmic staining on cancer cells of Human dysgerminoma of ovary is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

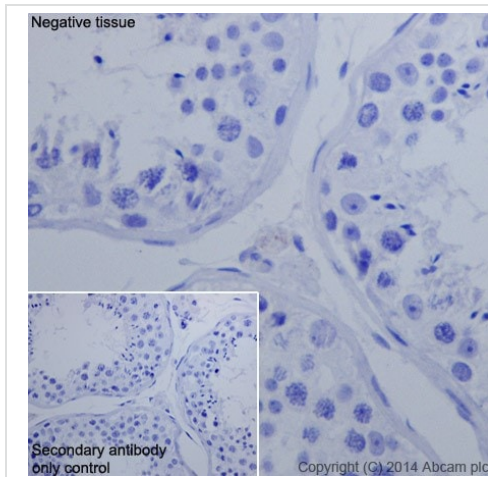


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Immunohistochemical analysis of paraffin-embedded Human breast cancer tissue labeling Oct4 with ab181557 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) secondary antibody at 1/500 dilution. Negative staining on Human breast cancer. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Oct4 antibody [EPR17929] - ChIP Grade (ab181557)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling Oct4 with ab181557 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) secondary antibody at 1/500 dilution. Negative staining on adult Human testis. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/500 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Oct4 antibody [EPR17929] - ChIP Grade
(ab181557)

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