# abcam

# Product datasheet

# Anti-c-Jun antibody [E254] - ChIP Grade ab32137

יולצעבע RabMAb

#### ★★★★ 4 Abreviews 95 References 画像数8

#### 製品の概要

製品名 Anti-c-Jun antibody [E254] - ChIP Grade

製品の詳細 Rabbit monoclonal [E254] to c-Jun - ChIP Grade

由来種 Rabbit

特異性 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

適用あり: WB, IHC-P, IP, ChIP アプリケーション

適用なし: Flow Cyt

交差種: Mouse, Rat, Human 種交差性

交差が予測される動物種: Pig 🔷

免疫原 Synthetic peptide within Human c-Jun aa 1-100 (N terminal). The exact sequence is proprietary.

エピトープ ab32137 reacts with an epitope located in the N terminal region of c-Jun.

ポジティブ・コントロール WB: NIH 3T3 and HeLa cell lysate. IHC-P: Human lung carcinoma tissue IP: HEK-293 and NIH3T3

cells.

特記事項 This 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**.

### 製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

 $K_D = 2.23 \times 10^{-11} M$ 解離定数(KD値)

10<sup>-11</sup>



# Learn more about K<sub>D</sub>

**バッファー** pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** E254 **アイソタイプ** IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32137の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	<b>★★★★★ (1)</b>	1/1000 - 1/10000. Predicted molecular weight: 36 kDa.
IHC-P	**** (2)	1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
IP		1/60.
ChIP		Use at an assay dependent concentration.

追加情報 Is unsuitable for Flow Cyt.

#### ターゲット情報

機能 Transcription factor that recognizes and binds to the enhancer heptamer motif 5'-TGA[CG]TCA-3'.

Promotes activity of NR5A1 when phosphorylated by HIPK3 leading to increased steroidogenic gene expression upon cAMP signaling pathway stimulation. Involved in activated KRAS-mediated transcriptional activation of USP28 in colorectal cancer (CRC) cells (PubMed:24623306). Binds

to the USP28 promoter in colorectal cancer (CRC) cells (PubMed:24623306).

**配列類似性** Belongs to the bZIP family. Jun subfamily.

Contains 1 bZIP (basic-leucine zipper) domain.

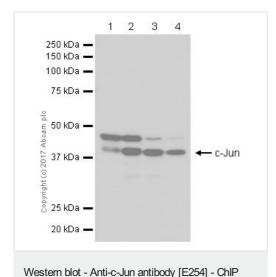
翻訳後修飾 Ubiquitinated by the SCF(FBXW7), leading to its degradation. Ubiquitination takes place

following phosphorylation, that promotes interaction with FBXW7.

Phosphorylated by CaMK4 and PRKDC; phosphorylation enhances the transcriptional activity. Phosphorylated by HIPK3. Phosphorylated by DYRK2 at Ser-243; this primes the protein for subsequent phosphorylation by GSK3B at Thr-239. Phosphorylated at Thr-239, Ser-243 and Ser-249 by GSK3B; phosphorylation reduces its ability to bind DNA. Phosphorylated by PAK2 at Thr-2, Thr-8, Thr-89, Thr-93 and Thr-286 thereby promoting JUN-mediated cell proliferation and transformation. Phosphorylated by PLK3 following hypoxia or UV irradiation, leading to increase

Grade (ab32137)

### 画像



**All lanes :** Anti-c-Jun antibody [E254] - ChIP Grade (ab32137) at 1/200 dilution

**Lane 1 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates prepared in RIPA lysis method

**Lane 2 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates prepared in 1% SDS Hot lysis method.

Lane 3: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates prepared in RIPA lysis method

**Lane 4**: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates prepared in 1% SDS Hot lysis method 15ug.

Lysates/proteins at 15 µg per lane.

## Secondary

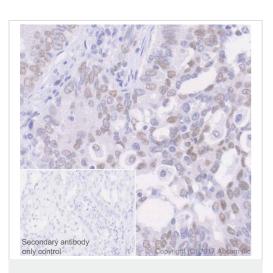
**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

**Predicted band size:** 36 kDa **Observed band size:** 39 kDa

Exposure time: 3 minutes

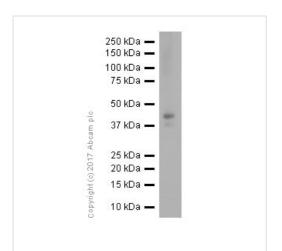
Blocking and diluting buffer: 5% NFDM/TBST

For Lysate preparation protocol, please refer to the protocol book in the protocol section and/or **here (downloadable copy)**.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue sections labeling c-Jun with purified ab32137 at 1:100 dilution (1.2 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



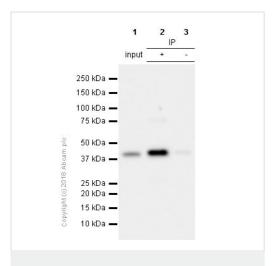
Western blot - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137)

Anti-c-Jun antibody [E254] - ChIP Grade (ab32137) at 1/5000 dilution + PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 20  $\mu g$ 

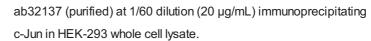
# Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 36 kDa Observed band size: 43 kDa



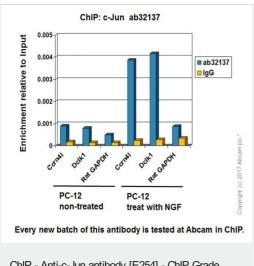
Immunoprecipitation - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137)



Lane 2 (+): HEK-293(Human embronic kidney epithelial cell) whole cell lysate 10  $\mu g$ 

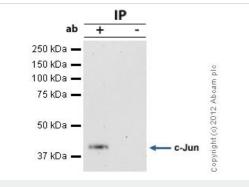
Lane 3 (-): ab32137 & HEK-293 whole cell lysateRabbit monoclonal lgG (ab172730) instead of ab32137 in HEK-293 whole cell lysate For western blotting, ab32137 at 1/500 dilution (2.29  $\mu$ g/mL) and VeriBlot for IP Detection Reagent (HRP) (ab131366) was used for detection at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM /TBST.



ChIP - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137)

Chromatin was prepared from PC-12 (starve overnight) + NGF (50 ng/ml 2h) cells according to the Abcam X-ChIP protocol. Cells were fixed with 1% formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab32137 (blue), and 20µl of protein A/G sepharose beads slurry (10µl of sepharose A beads + 10µl of sepharose G beads). 5µg of rabbit normal lgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Immunoprecipitation - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137)

c-Jun Probe Conc E254 \_\_\_ 99 nM 14.0 \_\_\_\_ 33 nM 12.0 Surface Density (ng/mm²) \_\_\_\_ 11 nM 10.0 \_\_\_ 3.7 nM 8.0 6.0 kon [1/ (sec-M)] =  $(1.008 \pm 0.004)$  E5 koff [1/sec] =  $(2.25 \pm 0.08)$  E-6 4.0 koff [1/sec] =  $(2.25 \pm 0.08)$  E Kd [M] =  $(2.23 \pm 0.08)$  E-11 2.0 Fit RMSE [ng/mm²] = 1.8 E-1
Median Baseline Noise [ng/mm²] = 5.8 E-2
Median Association Signal [ng/mm²] = 6.5 E0
Median Assoc. Endpoint SNR = 1.1 E2
RMSE / Noise = 3.0 E0 0.0 -2.0 30 60 90 135 Time (min)

OI-RD Scanning - Anti-c-Jun antibody [E254] - ChIP Grade (ab32137) c-Jun was immunoprecipitated using 0.5mg NIH3T3 whole cell extract, 5µg of Rabbit polyclonal to c-Jun and 50µl of protein G magnetic beads (+). No antibody was added to the control (-). The antibody was incubated under agitation with Protein G beads for 10min, NIH3T3 whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

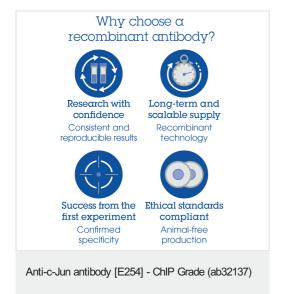
Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab32137.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit lgG light chain (HRP) (ab99697).

Band: 45kDa; c-Jun

Equilibrium disassociation constant ( $K_D$ ) Learn more about  $K_D$ 

Click here to learn more about K<sub>D</sub>



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