**Overview**

**Product name**
Anti-c-Jun antibody [E254] ab32137

**Description**
Rabbit monoclonal [E254] to c-Jun

**Host species**
Rabbit

**Tested applications**
Suitable for: WB, IHC-P, IP, ICC/IF
Unsuitable for: Flow Cyt

**Species reactivity**
Reacts with: Mouse, Rat, Human

**Predicted to work with**: Pig

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

**Epitope**
ab32137 reacts with an epitope located in the N terminal region of c-Jun.

**Positive control**
Skin carcinoma, NIH 3T3 cells. HeLa cell line (IF/ICC)

**General notes**
A trial size is available to purchase for this antibody.

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

This product is a recombinant rabbit monoclonal antibody.

**Properties**

**Form**
Liquid

**Storage instructions**
Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

**Dissociation constant (K_D)**
K_D = 2.23 x 10^-11 M

**Storage buffer**
pH: 7.20
Preservative: 0.01% Sodium azide
Constituents: 9% PBS, 40% Glycerol, 0.05% BSA, 50% Tissue culture supernatant

Purity
Tissue culture supernatant

Clonality
Monoclonal

Clone number
E254

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab32137 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>WB</td>
<td></td>
<td>1/1000 - 1/10000. Predicted molecular weight: 36 kDa.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/250.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use a concentration of 5 µg/ml.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/250.</td>
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Application notes
Is unsuitable for Flow Cyt.

Target

Function
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).

Sequence similarities
Belongs to the bZIP family. Jun subfamily.
Contains 1 bZIP (basic-leucine zipper) domain.

Post-translational modifications
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 DNA-binding activity.
Acetylated at Lys-271 by EP300.

Cellular localization
Nucleus.

Images
Anti-c-Jun antibody [E254] (ab32137) at 1/2000 dilution + NIH 3T3 cell lysate

**Predicted band size:** 36 kDa  
**Observed band size:** 40 kDa

Immunohistochemical analysis of c-Jun expression in paraffin embedded skin carcinoma tissue sample, using 1/250 ab32137.
**Western blot** - Anti-c-Jun antibody [E254] (ab32137)

**All lanes**: Anti-c-Jun antibody [E254] (ab32137) at 1/2000 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) (ab97051) 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).
Immunocytochemistry/ Immunofluorescence - Anti-c-Jun antibody [E254] (ab32137) staining c-Jun in HeLa cells treated with curcumin (diferuloylmethane) (ab120618), by ICC/IF. Decrease in c-Jun expression correlates with increased concentration of curcumin (diferuloylmethane) as described in literature.

The cells were incubated at 37°C for 4h in media containing different concentrations of ab120618 (curcumin (diferuloylmethane)) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab32137 (1/100 dilution) was performed overnight at 4°C in PBS containing 1% BSA. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

Immunoprecipitation - Anti-c-Jun antibody [E254] (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.


Band: 45kDa; c-Jun
ICC/IF image of ab32137 stained HeLa cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32137, 1/200 dilution) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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