Product name: Anti-IRE1 (phospho S724) antibody

Description: Rabbit polyclonal to IRE1 (phospho S724)

Host species: Rabbit

Specificity: ab48187 detects IRE1 alpha, phosphorylated (serine 724) protein.

Tested applications: Suitable for: WB, IHC-P, ELISA, IP, IHC-Fr, ICC/IF

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

Does not react with: Monkey

Immunogen: Synthetic peptide corresponding to Human IRE1 (phospho S724).

Database link: O75460

(Peptide available as ab110445)

Positive control: Glucose treated pancreatic islets and insulinoma cells.

Form: Liquid

Storage instructions: Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Storage buffer: Preservative: 0.02% Sodium azide

Constituent: PBS

Purity: Immunogen affinity purified

Clonality: Polyclonal

Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab48187 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
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<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐⭐</td>
<td>1/1000 - 1/2000. Predicted molecular weight: 110 kDa. Block with 3-5% BSA.</td>
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<tr>
<td>IHC-P</td>
<td></td>
<td>1/10 - 1/500.</td>
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<tr>
<td>ELISA</td>
<td></td>
<td>1/100 - 1/2000.</td>
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<tr>
<td>IP</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 20477942</td>
</tr>
</tbody>
</table>

**Target**

**Function**
Senses unfolded proteins in the lumen of the endoplasmic reticulum via its N-terminal domain which leads to enzyme auto-activation. The active endoribonuclease domain splices XBP1 mRNA to generate a new C-terminus, converting it into a potent unfolded-protein response transcriptional activator and triggering growth arrest and apoptosis.

**Tissue specificity**
Ubiquitously expressed. High levels observed in pancreatic tissue.

**Sequence similarities**
Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. Contains 1 KEN domain. Contains 1 protein kinase domain.

**Post-translational modifications**
Autophosphorylated.

**Cellular localization**
Endoplasmic reticulum membrane.
All lanes : Anti-IRE1 (phospho S724) antibody (ab48187) at 1/2000 dilution

Lane 1 : HeLa cells 30 nM Calyculin A: AP-buffer
Lane 2 : HeLa cells 30 nM Calyculin A: Alkaline Phosphatase

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : infrared (IR)-labelled goat anti-rabbit (green) antibody and IR-labelled goat anti-mouse (red) at 1/10000 dilution

Performed under reducing conditions.

Predicted band size: 110 kDa

The blots were produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membranes were blocked for an hour. Membrane 2 was incubated with alkaline phosphatase (AP; 100 U per mL) for one hour, whilst membrane 1 was treated with AP-buffer only, before being incubated with ab48187 (rabbit anti-IRE1 antibody diluted 1:2000) and loading control ab125247 (mouse anti-GAPDH antibody; diluted 1:10,000) for 24 hours at 4°C. Antibody binding was detected using infrared (IR)-labelled goat anti-rabbit (green) antibody and IR-labelled goat anti-mouse (red) at 1:10,000 dilutions for 1 hour at room temperature before imaging.
ab48187 staining IRE1 (phospho S724) in rat testis tissue sections by Immunohistochemistry (frozen sections). Tissue was fixed with formaldehyde, permeabilized using 0.1% Triton X-100 and then blocked with 2% BSA for 1 hour at 25°C, followed by incubation with the primary antibody at a 1/200 dilution, for 1 hour at 25°C. The secondary antibody used was a goat anti-rabbit IgG conjugated to Alexa Fluor® 488 (green) used at a 1/500 dilution.

Serially diluted ab48187 was bound to immobilised Phospho peptide (133861) - or Control peptide (1 microgram x mL-1). The antibody was detected by HRP-labelled goat anti-rabbit IgG (ab97080; diluted 50000 times) and signal was developed with TMB substrate.

All lanes: Anti-IRE1 (phospho S724) antibody (ab48187) at 1/1000 dilution

Lane 1: Min6 cells untreated
Lane 2: Min6 cells treated with glucose for 3 hours at 5 mM
Lane 3: Min6 cells treated with glucose for 3 hours at 20 mM

Developed using the ECL technique.

Predicted band size: 110 kDa
**All lanes**: Anti-IRE1 (phospho S724) antibody (ab48187) at 1/2000 dilution

**Lane 1**: Cell lysate prepared from COS-7 Untransfected cells

**Lane 2**: Cell lysate prepared from COS-7 cells expressing wild type IRE1 alpha

**Lane 3**: Cell lysate prepared from COS-7 cells expressing kinase-dead IRE1 alpha

**Predicted band size**: 110 kDa

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**Please note**: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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