# abcam

### Product datasheet

## Anti-IRE1 (phospho S724) antibody ab48187

\*\*\*\*\* 19 Abreviews 257 References 6 Images

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Product name	Anti-IRE1 (phospho S724) antibody	
Description	Rabbit polyclonal to IRE1 (phospho S724)	
Host species	Rabbit	
Tested applications	Suitable for: WB, ELISA, IHC-P	
Species reactivity	Reacts with: Mouse, Human, Recombinant fragment	
Immunogen	Synthetic peptide corresponding to Human IRE1 (phospho S724). Database link: <u>075460</u> (Peptide available as <u>ab110445</u> )	
Positive control	WB: HeLa, Min6, transfected COS-7 cells. IHC-P: Human spleen tissue.	
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.	
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As	
Properties		
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	pH: 7.40 Preservative: 0.025% Sodium azide Constituent: PBS	
Purity	Immunogen affinity purified	
Clonality	Polyclonal	
lsotype	lgG	

#### The Abpromise guarantee

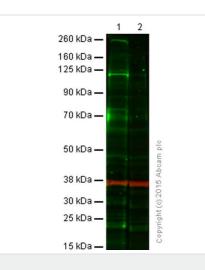
Our Abpromise guarantee covers the use of ab48187 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★	1/1000 - 1/2000. Predicted molecular weight: 110 kDa. Block with 3-5% BSA.
ELISA		1/100 - 1/2000.
IHC-P	<b>★ ★ ★ ★ ★ (1)</b>	1/300.

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.

#### Images



Western blot - Anti-IRE1 (phospho S724) antibody (ab48187) **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.

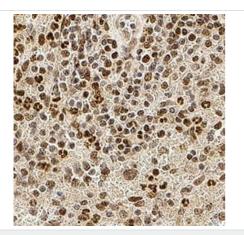
#### Secondary

**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.

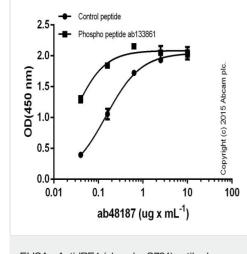
Predicted band size: 110 kDa

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.



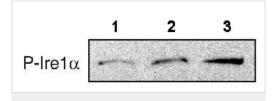
Paraffin-embedded human spleen tissue stained for IRE1 using ab48187 at 1/300 dilution for 1 hour at room temperature in immunohistochemical analysis. DAB staining. Counter stained with hematoxylin.

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-IRE1 (phospho S724) antibody (ab48187)



ELISA - Anti-IRE1 (phospho S724) antibody (ab48187)

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 lgG (<u>ab97080</u>; diluted 50000 times) and signal was developed with TMB substrate.



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

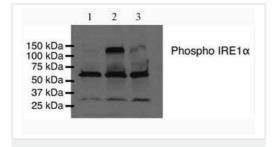
Lane 1 : Min6 cells untreated

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

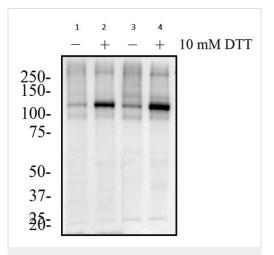


Western blot - Anti-IRE1 (phospho S724) antibody (ab48187) **All lanes :** Anti-IRE1 (phospho S724) antibody (ab48187) at 1/2000 dilution

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 kinasedead IRE1 alpha

Predicted band size: 110 kDa



Western blot - Anti-IRE1 (phospho S724) antibody (ab48187) All lanes : Anti-IRE1 (phospho S724) antibody (ab48187) at 2 µg/ml

Lane 1 : Untreated HeLa cells (Batch 1 ab48187)

Lane 2 : DTT-treated HeLa cells (Batch 1 ab48187)

Lane 3 : Untreated HeLa cells (Batch 2 ab48187)

Lane 4 : DTT-treated HeLa cells (Batch 2 ab48187)

#### Secondary

All lanes : Anti-Rabbit lgG HRP

Predicted band size: 110 kDa

HeLa cells were treated (+) or untreated (-) with 10 mM DTT for 60 min to activate the UPR. Total protein was separated on a 7.5% gel by SDS-PAGE, transferred to PVDF membrane and blocked in 5% BSA in TBST.

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