Product datasheet

Anti-Insulin Receptor alpha antibody [83-7] ab36550

Overview

Product name  Anti-Insulin Receptor alpha antibody [83-7]
Description  Mouse monoclonal [83-7] to Insulin Receptor alpha
Host species  Mouse
Specificity  Does not cross react with the Human Type 1 IGF Receptor.
Tested applications  Suitable for: Flow Cyt, IP, ELISA, IHC-P
Species reactivity  Reacts with: Human
Does not react with: Rat
Immunogen  Transfected 3T3 cells overexpressing the human Insulin Receptor alpha
Epitope  ab36550 recognizes an epitope within amino acids 140-301 (the cysteine rich region) of the extracellular domain of the human Insulin Receptor alpha.

General notes  The antibody enhances the binding of $^{125}$I insulin binding to the insulin receptor of HIR3.5/3T3 cells and stimulates insulin mediated $^3$H thymidine incorporation in these cells.

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  Constituent: PBS
Purity  Protein G purified
Primary antibody notes  The antibody enhances the binding of $^{125}$I insulin binding to the insulin receptor of HIR3.5/3T3 cells and stimulates insulin mediated $^3$H thymidine incorporation in these cells.
Clonality  Monoclonal
Clone number  83-7
Isotype  IgG1

Applications
Relevance

The human insulin receptor is a heterotetrameric membrane glycoprotein consisting of disulfide linked subunits in a beta-alpha-alpha-beta configuration. The beta subunit (95 kDa) possesses a single transmembrane domain, whereas the alpha subunit (135 kDa) is completely extracellular. The insulin receptor exhibits receptor tyrosine kinase (RTK) activity. RTKs are single pass transmembrane receptors that possess intrinsic cytoplasmic enzymatic activity, catalyzing the transfer of the gamma phosphate of ATP to tyrosine residues in protein substrates. RTKs are essential components of signal transduction pathways that affect cell proliferation, differentiation, migration and metabolism. Included in this large protein family are the insulin receptor and the receptors for growth factors such as epidermal growth factor, fibroblast growth factor and vascular endothelial growth factor. Receptor activation occurs through ligand binding, which facilitates receptor dimerization and autophosphorylation of specific tyrosine residues in the cytoplasmic portion. The interaction of insulin with the alpha subunit of the insulin receptor activates the protein tyrosine kinase of the beta subunit, which then undergoes an autophosphorylation that increases its tyrosine kinase activity. Three adapter proteins, IRS1, IRS2 and Shc, become phosphorylated on tyrosine residues following insulin receptor activation. These three phosphorylated proteins then interact with SH2 domain containing signaling proteins.

Cellular localization

Membrane; single pass type I membrane protein.

Our Abpromise guarantee covers the use of ab36550 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>
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<tbody>
<tr>
<td>Flow Cyt</td>
<td>Use 1µg for 10⁶ cells. (paraformaldehyde or methanol fixed cells)</td>
<td></td>
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<tr>
<td>IP</td>
<td>1/5000.</td>
<td></td>
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<tr>
<td>ELISA</td>
<td>1/5000.</td>
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<tr>
<td>IHC-P</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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</tbody>
</table>

Target

Relevance

The human insulin receptor is a heterotetrameric membrane glycoprotein consisting of disulfide linked subunits in a beta-alpha-alpha-beta configuration. The beta subunit (95 kDa) possesses a single transmembrane domain, whereas the alpha subunit (135 kDa) is completely extracellular. The insulin receptor exhibits receptor tyrosine kinase (RTK) activity. RTKs are single pass transmembrane receptors that possess intrinsic cytoplasmic enzymatic activity, catalyzing the transfer of the gamma phosphate of ATP to tyrosine residues in protein substrates. RTKs are essential components of signal transduction pathways that affect cell proliferation, differentiation, migration and metabolism. Included in this large protein family are the insulin receptor and the receptors for growth factors such as epidermal growth factor, fibroblast growth factor and vascular endothelial growth factor. Receptor activation occurs through ligand binding, which facilitates receptor dimerization and autophosphorylation of specific tyrosine residues in the cytoplasmic portion. The interaction of insulin with the alpha subunit of the insulin receptor activates the protein tyrosine kinase of the beta subunit, which then undergoes an autophosphorylation that increases its tyrosine kinase activity. Three adapter proteins, IRS1, IRS2 and Shc, become phosphorylated on tyrosine residues following insulin receptor activation. These three phosphorylated proteins then interact with SH2 domain containing signaling proteins.

Cellular localization

Membrane; single pass type I membrane protein.

Images
ab36550 (1µg/ml) staining insulin receptor alpha in human pancreas using an automated system (DAKO Autostainer Plus).

Using this protocol there is strong staining of cytoplasm and basal cell membrane of proximal convoluted tubule cells.

Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH 6.0 in a DAKO PT link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.

Overlay histogram showing Jurkat cells stained with ab36550 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab36550, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Jurkat cells fixed with methanol (5 min) used under the same conditions.

Please note that Abcam do not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.

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