Overview

Product name: Human EphA2 ELISA Kit
Detection method: Colorimetric

Precision

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>8</td>
<td></td>
<td></td>
<td>2.6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3</td>
<td></td>
<td></td>
<td>4.2%</td>
</tr>
</tbody>
</table>

Sample type: Cell culture extracts
Assay type: Sandwich (quantitative)
Sensitivity: 0.88 pg/ml
Range: 9.4 pg/ml - 600 pg/ml

Recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture extracts</td>
<td>108</td>
<td>105% - 113%</td>
</tr>
</tbody>
</table>

Assay time: 1h 30m
Assay duration: One step assay

Species reactivity: Reacts with: Human

Product overview

EphA2 in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of EphA2 protein in cell extracts.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire
complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

Notes

Ephrin type-A Receptor 2, EPHA2, is a receptor tyrosine kinase encoded by the gene EPHA2. EphA2 is a 976-amino acid containing transmembrane glycoprotein, and is a member of the ephrin receptor subfamily of the protein-tyrosine kinase family. The extracellular portion of EphA2 contains the ephrin-A ligand binding site, and two fibronectin type-III repeats. EphA2 regulates cell adhesion and differentiation and is thought to be participate in mediating developmental events, especially in the nervous system. EphA2 has been shown to be highly expressed in tumor cell lines.

Platform

Reagents

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>1 x 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Wash Buffer PT (ab206977)</td>
<td>1 x 20ml</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution (ab193971)</td>
<td>1 x 1ml</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR (ab193970)</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Antibody Diluent 5BI</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>10X Human EphA2 Capture Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>10X Human EphA2 Detector Antibody</td>
<td>1 x 600µl</td>
</tr>
<tr>
<td>Human EphA2 Lyophilized Recombinant Protein</td>
<td>2 vials</td>
</tr>
<tr>
<td>Plate Seals</td>
<td>1 unit</td>
</tr>
<tr>
<td>Sample Diluent NS (ab193972)</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>SimpleStep Pre-Coated 96-Well Microplate (ab206978)</td>
<td>1 unit</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 12ml</td>
</tr>
<tr>
<td>TMB Development Solution</td>
<td>1 x 12ml</td>
</tr>
</tbody>
</table>

Function

Receptor for members of the ephrin-A family. Binds to ephrin-A1, -A3, -A4 and -A5. Plays an important role in angiogenesis and tumor neovascularization. The recruitment of VAV2, VAV3 and P3-kinase p85 subunit by phosphorylated EPHA2 is critical for EFNA1-induced RAC1 GTPase activation and vascular endothelial cell migration and assembly (By similarity). Induces
apoptosis in a p53/TP53-independent, caspase-8-dependent manner.

**Tissue specificity**
Expressed in brain and glioma tissue and glioma cell lines (at protein level). Expressed most highly in tissues that contain a high proportion of epithelial cells, e.g., skin, intestine, lung, and ovary.

**Involvement in disease**
Genetic variations in EPHA2 are the cause of susceptibility to cataract cortical age-related type 2 (ARCC2) [MIM:613020]. A developmental punctate opacity common in the cortex and present in most lenses. The cataract is white or cerulean, increases in number with age, but rarely affects vision.
Defects in EPHA2 are the cause of cataract posterior polar type 1 (CTPP1) [MIM:116600]. A subcapsular opacity, usually disk-shaped, located at the back of the lens. It can have a marked effect on visual acuity.

**Sequence similarities**
Belongs to the protein kinase superfamily. Tyr protein kinase family. Ephrin receptor subfamily.
Contains 2 fibronectin type-III domains.
Contains 1 protein kinase domain.
Contains 1 SAM (sterile alpha motif) domain.

**Post-translational modifications**
Activated by EFNA1 via tyrosine phosphorylation. Phosphorylated residues Tyr-588 and Tyr-594 are required for binding VAV2 and VAV3 while phosphorylated residues Tyr-735 and Tyr-930 are required for binding PI3-kinase p85 subunit. These phosphorylated residues are critical for recruitment of VAV2 and VAV3 and PI3-kinase p85 subunit which transduce downstream signaling to activate RAC1 GTPase and endothelial cell migration. They also play a critical role in transducing EPHA2 signaling in vascular endothelial cells during tumor angiogenesis.

**Cellular localization**
Membrane.

**SimpleStep ELISA technology**
SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.

**Background-subtracted data values**
Background-subtracted data values (mean +/- SD) are graphed.
The concentrations of EphA2 were measured in duplicate, interpolated from the EphA2 standard curve, and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean EphA2 concentration was determined to be 940 pg/mL in HepG2 cell extract, 1,076 pg/mL in HEK293T cell extract, and 17.4 ng/mL in PC-3 cell extract.

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