Human Agrin ELISA Kit ab216945

Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Human Agrin ELISA Kit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection method</td>
<td>Colorimetric</td>
</tr>
</tbody>
</table>

### Precision

#### Intra-assay

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

#### Inter-assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>5</td>
<td></td>
<td></td>
<td>5.7%</td>
</tr>
</tbody>
</table>

### Sample type

- Cell culture supernatant
- Urine
- Serum
- Cell culture extracts
- Tissue Extracts
- Hep Plasma
- EDTA Plasma
- Cit plasma
- Cerebral Spinal Fluid

### Assay type

- Sandwich (quantitative)

### Sensitivity

- 4.6 pg/ml

### Range

- 28.13 pg/ml - 1800 pg/ml

### Recovery

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Average %</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell culture supernatant</td>
<td>119</td>
<td>116% - 121%</td>
</tr>
<tr>
<td>Urine</td>
<td>106</td>
<td>101% - 114%</td>
</tr>
<tr>
<td>Serum</td>
<td>97</td>
<td>87% - 113%</td>
</tr>
<tr>
<td>Cell culture extracts</td>
<td>98</td>
<td>90% - 103%</td>
</tr>
<tr>
<td>Tissue Extracts</td>
<td>118</td>
<td>115% - 122%</td>
</tr>
<tr>
<td>Hep Plasma</td>
<td>108</td>
<td>101% - 112%</td>
</tr>
<tr>
<td>Sample type</td>
<td>Average %</td>
<td>Range</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>106</td>
<td>104% - 109%</td>
</tr>
<tr>
<td>Cit plasma</td>
<td>113</td>
<td>97% - 121%</td>
</tr>
<tr>
<td>Cerebral Spinal Fluid</td>
<td>106</td>
<td>86% - 126%</td>
</tr>
</tbody>
</table>

**Assay time**

1h 30m

**Assay duration**

One step assay

**Species reactivity**

Reacts with: Human

Does not react with: Cow

**Product overview**

Human Agrin ELISA Kit (ab216945) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Agrin protein in cell culture extracts, cell culture supernatant, cerebral spinal fluid, cit plasma, edta plasma, hep plasma, serum, tissue extracts, and urine. It uses our proprietary SimpleStep ELISA® technology. Quantitate Human Agrin with 4.6 pg/ml sensitivity.

SimpleStep ELISA® technology employs capture antibodies conjugated to an affinity tag that is recognized by the monoclonal antibody used to coat our SimpleStep ELISA® plates. This approach to sandwich ELISA allows the formation of the antibody-analyte sandwich complex in a single step, significantly reducing assay time. See the SimpleStep ELISA® protocol summary in the image section for further details. Our SimpleStep ELISA® technology provides several benefits:

- Single-wash protocol reduces assay time to 90 minutes or less
- High sensitivity, specificity and reproducibility from superior antibodies
- Fully validated in biological samples
- 96-wells plate breakable into 12 x 8 wells strips

A 384-well SimpleStep ELISA® microplate (ab203359) is available to use as an alternative to the 96-well microplate provided with SimpleStep ELISA® kits.

**Notes**

Agrin is a heparan sulfate basal lamina glycoprotein that plays a central role in the formation and the maintenance of the neuromuscular junction (NMJ) and directs key events in postsynaptic differentiation. Agrin is a component of the AGRN-LRP4 receptor complex that induces the phosphorylation and activation of MUSK. The activation of MUSK in myotubes induces the formation of NMJ by regulating different processes including the transcription of specific genes and the clustering of AChR in the postsynaptic membrane. Calcium ions are required for maximal AChR clustering. Agrin function in neurons is highly regulated by alternative splicing, glycan binding and proteolytic processing. Agrin modulates calcium ion homeostasis in neurons, specifically by inducing an increase in cytoplasmic calcium ions. Agrin functions differentially in the central nervous system (CNS) by inhibiting the alpha(3)-subtype of Na+/K+-ATPase and evoking depolarization at CNS synapses.

Abcam has not and does not intend to apply for the REACH Authorisation of customers’ uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.
Platform
Pre-coated microplate (12 x 8 well strips)

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 Human Agrin Capture Antibody</td>
<td>1 x 600μl</td>
</tr>
<tr>
<td>10X Human Agrin Detector Antibody</td>
<td>1 x 600μl</td>
</tr>
<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 4BI</td>
<td>1 x 6ml</td>
</tr>
<tr>
<td>Human Agrin 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 50ml</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
Isoform 1: heparan sulfate basal lamina glycoprotein that plays a central role in the formation and the maintenance of the neuromuscular junction (NMJ) and directs key events in postsynaptic differentiation. Component of the AGRN-LRP4 receptor complex that induces the phosphorylation and activation of MUSK. The activation of MUSK in myotubes induces the formation of NMJ by regulating different processes including the transcription of specific genes and the clustering of AChR in the postsynaptic membrane. Calcium ions are required for maximal AChR clustering. AGRN function in neurons is highly regulated by alternative splicing, glycan binding and proteolytic processing. Modulates calcium ion homeostasis in neurons, specifically by inducing an increase in cytoplasmic calcium ions. Functions differentially in the central nervous system (CNS) by inhibiting the alpha(3)-subtype of Na+/K+-ATPase and evoking depolarization at CNS synapses. This secreted isoform forms a bridge, after release from motor neurons, to basal lamina through binding laminin via the NtA domain.

Isoform 2: transmembrane form that is the predominant form in neurons of the brain, induces dendritic filopodia and synapse formation in mature hippocampal neurons in large part due to the attached glycosaminoglycan chains and the action of Rho-family GTPases.

Isoform 1, isoform 4 and isoform 5: neuron-specific (z+) isoforms that contain C-terminal insertions of 8-19 AA are potent activators of AChR clustering. Isoform 5, agrin (z+8), containing the 8-AA insert, forms a receptor complex in myotubules containing the neuronal AGRN, the muscle-specific kinase MUSK and LRP4, a member of the LDL receptor family. The splicing factors, NOVA1 and NOVA2, regulate AGRN splicing and production of the ‘z’ isoforms.
Isoform 3 and isoform 6: lack any 'z' insert, are muscle-specific and may be involved in endothelial cell differentiation.

Agrin N-terminal 110 kDa subunit: is involved in regulation of neurite outgrowth probably due to the presence of the glycosaminoglycan (GAG) side chains of heparan and chondroitin sulfate attached to the Ser/Thr- and Gly/Ser-rich regions. Also involved in modulation of growth factor signaling.

Agrin C-terminal 22 kDa fragment: this released fragment is important for agrin signaling and to exert a maximal dendritic filopodia-inducing effect. All 'z' splice variants (z+) of this fragment also show an increase in the number of filopodia.

### Tissue specificity
Expressed in basement membranes of lung and kidney. Muscle- and neuron-specific isoforms are found. Isoforms (y+) with the 4 AA insert and (z+8) isoforms with the 8 AA insert are all neuron-specific. Isoforms (z+11) are found in both neuronal and non-neuronal tissues.

### Involvement in disease
Myasthenic syndrome, congenital, 8

### Sequence similarities
Contains 4 EGF-like domains.
Contains 9 Kazal-like domains.
Contains 2 laminin EGF-like domains.
Contains 3 laminin G-like domains.
Contains 1 NtA (N-terminal agrin) domain.
Contains 1 SEA domain.

### Domain
The NtA domain, absent in TM-agrin, is required for binding laminin and connecting to basal lamina.
Both laminin G-like 2 (G2) and laminin G-like 3 (G3) domains are required for alpha-dystroglycan/DAG1 binding. G3 domain is required for C-terminal heparin, heparan sulfate and sialic acid binding.

### Post-translational modifications
Contains heparan and chondroitin sulfate chains and alpha-dystroglycan as well as N-linked and O-linked oligosaccharides. Glycosaminoglycans (GAGs), present in the N-terminal 110 kDa fragment, are required for induction of filopodia in hippocampal neurons. The first cluster (Gly/Ser-rich) for GAG attachment contains heparan sulfate (HS) chains and the second cluster (Ser/Thr-rich), contains chondroitin sulfate (CS) chains. Heparin and heparin sulfate binding in the G3 domain is independent of calcium ions. Binds heparin with a stoichiometry of 2:1. Binds sialic acid with a stoichiometry of 1:1 and binding requires calcium ions.
At synaptic junctions, cleaved at two conserved sites, alpha and beta, by neurotrypsin. Cleavage at the alpha-site produces the agrin N-terminal 110-kDa subunit and the agrin C-terminal 110-kDa subunit. Further cleavage of agrin C-terminal 110-kDa subunit at the beta site produces the C-terminal fragments, agrin C-terminal 90 kDa fragment and agrin C-terminal 22 kDa fragment. Excessive cleavage at the beta-site releases large amounts of the agrin C-terminal 22 kDa fragment leading to destabilization at the neuromuscular junction (NMJ).

### Cellular localization
Cell junction, synapse. Cell membrane and Secreted, extracellular space, extracellular matrix. Synaptic basal lamina at the neuromuscular junction.

### Images
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 (mean +/- SD) are graphed.
Example of human Agrin standard curve in 1X Cell Extraction Buffer PTR.

Background-subtracted data values (mean +/- SD) are graphed.

Example of human Agrin standard curve in HGDMEM + 10% FBS cell culture media.

Background-subtracted data values (mean +/- SD) are graphed.
The concentrations of Agrin were measured in duplicates, interpolated from the Agrin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 25%, plasma (citrate) 25%, plasma (heparin) 25% and plasma (EDTA) 25%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Agrin concentration was determined to be 3,719 pg/mL in neat serum, 3,562 pg/mL in neat plasma (citrate), 3,526 pg/mL in neat plasma (heparin), and 3,445 pg/mL in neat plasma (EDTA).

The concentrations of Agrin were measured in duplicates, interpolated from the Agrin standard curves and corrected for sample dilution. Undiluted samples are as follows: Urine 12.5%, cerebrospinal fluid 6.25%, HepG2 cell culture supernatant 100% and HeLa cell culture supernatant 100%. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Agrin concentration was determined to be 9,142 pg/mL in neat urine, 18,572 pg/mL in neat cerebrospinal fluid, 1,365 pg/mL in HepG2 cell culture supernatant, and 711.7 pg/mL in HeLa cell culture supernatant.
Interpolated concentrations of native Agrin in human brain tissue extract, human liver tissue extract, SH-SY5Y cell extract, and HepG2 cell extract based on extract loads of 300 µg/mL, 1000 µg/mL, 500 µg/mL, and 100 µg/mL, respectively. The concentrations of Agrin were measured in duplicate and interpolated from the Agrin standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Agrin concentration was determined to be 540 pg/mL in brain tissue extract, 740 pg/mL in liver tissue extract, 252 pg/mL in SH-SY5Y cell extract, and 796 pg/mL in HepG2 cell extract.

Serum from ten individual healthy human male donors was measured in duplicate.

Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Agrin concentration in neat samples was determined to be 3909 pg/mL with a range of 2879 – 5073 pg/mL.
To learn more about the advantages of recombinant antibodies see [here](#).

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