abcam

Product datasheet

Human Clusterin ELISA Kit ab174447

Recombinant SimpleStep ELISA

7 References 12 Images

Overview

Product name

Human Clusterin ELISA Kit

Detection method

Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%	
Serum	9			2.7%	

Inter-assay

Sample	n	Mean	SD	CV%
Serum	3			4.9%

Sample type

Cell culture supernatant, Saliva, Milk, Urine, Serum, Cell culture extracts, Hep Plasma, EDTA

Plasma, Cit plasma

Assay type

Sandwich (quantitative)

Sensitivity

22.5 pg/ml

Range

234.4 pg/ml - 15000 pg/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Cell culture supernatant	103	100% - 106%
Saliva	100	99% - 100%
Milk	99	97% - 101%
Urine	102	101% - 102%
Serum	101	98% - 103%
Cell culture extracts	105	96% - 112%

1

Sample type	Average %	Range
Hep Plasma	102	101% - 103%
EDTA Plasma	101	99% - 102%
Cit plasma	107	106% - 109%

Assay time

1h 30m

Assay duration

One step assay

Species reactivity

Reacts with: Human, Rhesus monkey

Does not react with: Cow

Product overview

Human Clusterin ELISA kit (ab174447) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of Clusterin protein in human serum, plasma, urine, saliva, milk, cell culture supernatant, and cell extracts. It uses our proprietary SimpleStep ELISA® technology. Quantitate human Clusterin with 36.1 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 (<u>ab203359</u>) is available to use as an alternative to the 96-well microplate provided with SimpeStep ELISA® kits.

Clusterin (CLU) is composed of an antiparallel, disulfide-linked heterodimer of an alpha chain and a beta chain that self-associate and can form higher oligomers. It is ubiquitously expressed in various tissues. Clusterin functions as an extracellular chaperone that prevents aggregation of nonnative proteins. It does not require ATP and does not refold proteins by itself. Clusterin does so by maintaining partially unfolded proteins in a state appropriate for subsequent refolding by other chaperones, such as HSPA8/HSC70. Clusterin prevents stress-induced aggregation of blood plasma proteins by inhibiting the formation of amyloid fibrils through APP, APOC2, B2M, CALCA, CSN3, SNCA and aggregation-prone LYZ variants (in vitro). It can bind to cell surface receptors and trigger internalization of the chaperone-client complex and subsequent lysosomal or proteasomal degradation.

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.

Pre-coated microplate (12 x 8 well strips)

Notes

Platform

Storage instructions

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

Components	1 x 96 tests
10X Human Clusterin Capture Antibody	1 x 600µl
10X Human Clusterin Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent 4BR	1 x 6ml
Human Clusterin Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 50ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

Function

Isoform 1 functions as extracellular chaperone that prevents aggregation of nonnative proteins. Prevents stress-induced aggregation of blood plasma proteins. Inhibits formation of amyloid fibrils by APP, APOC2, B2M, CALCA, CSN3, SNCA and aggregation-prone LYZ variants (in vitro). Does not require ATP. Maintains partially unfolded proteins in a state appropriate for subsequent refolding by other chaperones, such as HSPA8/HSC70. Does not refold proteins by itself. Binding to cell surface receptors triggers internalization of the chaperone-client complex and subsequent lysosomal or proteasomal degradation. Secreted isoform 1 protects cells against apoptosis and against cytolysis by complement. Intracellular isoforms interact with ubiquitin and SCF (SKP1-CUL1-F-box protein) E3 ubiquitin-protein ligase complexes and promote the ubiquitination and subsequent proteasomal degradation of target proteins. Promotes proteasomal degradation of COMMD1 and IKBKB. Modulates NF-kappa-B transcriptional activity. Nuclear isoforms promote apoptosis. Mitochondrial isoforms suppress BAX-dependent release of cytochrome c into the cytoplasm and inhibit apoptosis. Plays a role in the regulation of cell proliferation.

Tissue specificity

Detected in blood plasma, cerebrospinal fluid, milk, seminal plasma and colon mucosa. Detected in the germinal center of colon lymphoid nodules and in colon parasympathetic ganglia of the Auerbach plexus (at protein level). Ubiquitous. Detected in brain, testis, ovary, liver and pancreas, and at lower levels in kidney, heart, spleen and lung.

Sequence similarities

Post-translational modifications

Belongs to the clusterin family.

lsoform 1 is proteolytically cleaved on its way through the secretory system, probably within the Golgi lumen.

Polyubiquitinated, leading to proteasomal degradation.

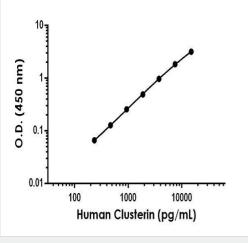
Heavily N-glycosylated. About 30% of the protein mass is comprised of complex N-linked carbohydrate.

Cellular localization

Secreted. Can retrotranslocate from the secretory compartments to the cytosol upon cellular

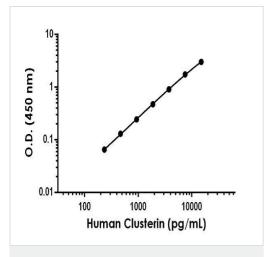
stress and Nucleus. Cytoplasm. Mitochondrion membrane. Cytoplasm, cytosol. Microsome. Endoplasmic reticulum. Cytoplasmic vesicle, secretory vesicle, chromaffin granule. Isoforms lacking the N-terminal signal sequence have been shown to be cytoplasmic and/or nuclear. Secreted isoforms can retrotranslocate from the secretory compartments to the cytosol upon cellular stress. Detected in perinuclear foci that may be aggresomes containing misfolded, ubiquitinated proteins. Detected at the mitochondrion membrane upon induction of apoptosis.

Images



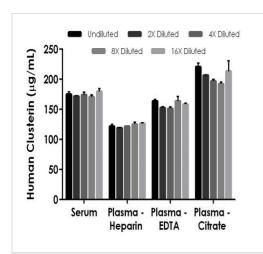
Example of human Clusterin standard curve in Sample Diluent NS.

The Clusterin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



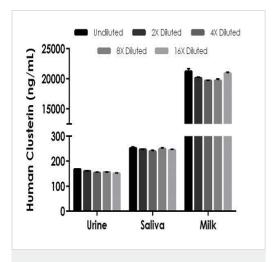
Example of human Clusterin standard curve in 1X Cell Extraction Buffer PTR.

The Clusterin standard curve was prepared as described in Section 10. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



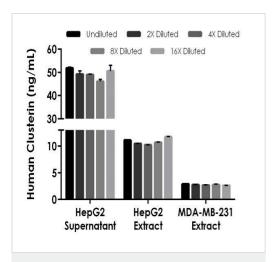
Interpolated concentrations of native Clusterin in human serum and plasma samples.

The concentrations of Clusterin were measured in duplicates, interpolated from the Clusterin standard curves and corrected for sample dilution. Undiluted samples are as follows: serum 1:80,000, plasma (heparin) 1:40,000, plasma (EDTA) 1:40,000, and plasma (citrate) 1:20,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Clusterin concentration in the neat samples was determined to be 174 μ g/mL in serum, 123 μ g/mL in plasma (heparin), 158 μ g/mL in plasma (EDTA), and 206 μ g/mL in plasma (citrate).



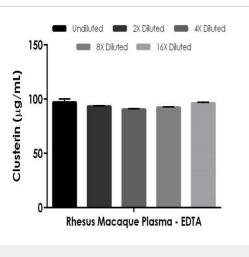
Interpolated concentrations of native Clusterin in human urine, saliva, and milk.

The concentrations of Clusterin were measured in duplicates, interpolated from the Clusterin standard curves and corrected for sample dilution. Undiluted samples are as follows: urine 1:32, saliva 1:80, and milk 1:3,200. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Clusterin concentration in the neat samples was determined to be 157 ng/mL in urine, 246 ng/mL in saliva, and 20,323 ng/mL in milk.



The concentrations of Clusterin were measured in duplicates, interpolated from the Clusterin standard curves and corrected for sample dilution. Undiluted samples are as follows: HepG2 supernatant 1:12, HepG2 extract 200 µg/mL, and MDA-MB-231 extract 750 µg/mL.

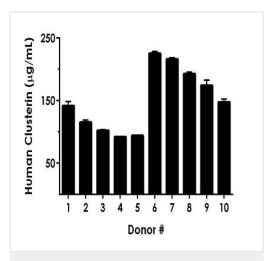
Interpolated concentrations of native Clusterin in human HepG2 cell supernatant, HepG2 cell extract, and MDA-MB-231 cell extract.



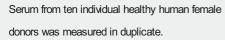
Rhesus Macaque plasma (EDTA).

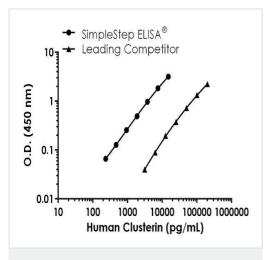
Interpolated concentrations of native Clusterin in

The concentrations of Clusterin were measured in duplicate, interpolated from the Clusterin standard curve and corrected for sample dilution. Undiluted samples are 1:20,000. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Clusterin concentration in the neat sample was determined to be 94 µg/mL.



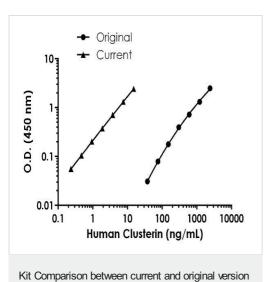
Interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean Clusterin concentration was determined to be 150 μ g/mL with a range of 92 – 227 μ g/mL.



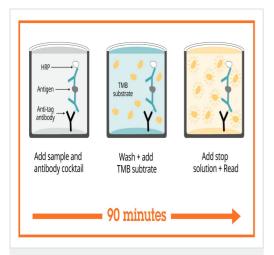


Human Clusterin Competitor Std Curve Comparison

Standard curve comparison between human Clusterin SimpleStep ${\sf ELISA}^{@}\ kit\ and\ traditional\ {\sf ELISA}\ kit\ from\ leading\ competitor.$ SimpleStep ELISA kit shows increased sensitivity.



Standard curve comparison between current human Clutsterin SimpleStep $ELISA^{@}$ kit and original human Clusterin SimpleStep $ELISA^{@}$ kit. The current SimpleStep ELISA kit shows increased sensitivity.



Sandwich ELISA - Human Clusterin ELISA Kit

(ab174447)

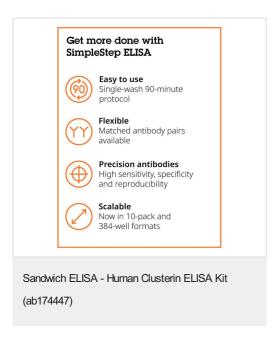
(ab174447)

SimpleStep ELISA technology allows the formation of the antibodyantigen 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.



Sandwich ELISA - Human Clusterin ELISA Kit

To learn more about the advantages of recombinant antibodies see **here**.



To learn more about the advantages of SimpleStep ELISA® kits see <u>here</u>.

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