abcam

Product datasheet

Human AIF ELISA Kit (Apoptosis-Inducing Factor 1) ab184858

SimpleStep ELISA

4 Images

Overview

Product name

Human AIF ELISA Kit (Apoptosis-Inducing Factor 1)

Detection method

Colorimetric

Precision

Sample	n	Mean	SD	CV%
Hel a extract	5			1 9%

Inter-assay

Intra-assay

Sample	n	Mean	SD	CV%	
HeLa extract	3			3.2%	

Sample type

Serum, Plasma, Cell culture extracts, Tissue Extracts

Assay type

Sandwich (quantitative)

Sensitivity

60 pg/ml

Range

0.156 ng/ml - 10 ng/ml

Recovery

Sample specific recovery

Sample type	Average %	Range
Serum	103.2	102% - 105.6%
Cell culture media	99	97.3% - 101%
Hep Plasma	107	105.6% - 108.4%
EDTA Plasma	105.3	104.1% - 107.3%
Cit plasma	106.2	101.7% - 110%

1

Assay time 1h 30m

Assay duration One step assay

Species reactivity Reacts with: Human

Product overview

Human AIF (Apoptosis-Inducing Factor 1) ELISA kit (ab184858) is a single-wash 90 min sandwich ELISA designed for the quantitative measurement of AIF protein in human serum, plasma, cell and tissue extract samples. It uses our proprietary SimpleStep ELISA® technology. Quantitate human AIF with 60 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.

AIF functions both as NADH oxidoreductase and as regulator of apoptosis. In response to apoptotic stimuli, it is released from the mitochondrion intermembrane space into the cytosol and to the nucleus, where it functions as a proapoptotic factor in a caspase-independent pathway. In contrast, AIF functions as an antiapoptotic factor in normal mitochondria via its NADH oxidoreductase activity. The soluble form of AIF (AIFsol) is found in the nucleus induces 'parthanatos' i.e. caspase-independent fragmentation of chromosomal DNA. AIF interacts with EIF3G, and thereby inhibits the EIF3 machinery and protein synthesis, and activates casapse-7 to amplify apoptosis. AIF plays a critical role in caspase-independent, pyknotic cell death in hydrogen peroxide-exposed cells. AIF binds to DNA in a sequence-independent manner.

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 Microplate

Properties

Storage instructions

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

Components	1 x 96 tests
10X Human AIF Detector Antibody	1 x 600µl
10X Wash Buffer PT (ab206977)	1 x 20ml
50X Cell Extraction Enhancer Solution (ab193971)	1 x 1ml

Notes

Components	1 x 96 tests
5X Cell Extraction Buffer PTR (ab193970)	1 x 10ml
Antibody Diluent CPI - HAMA Blocker (ab193969)	1 x 6ml
Human AIF Capture Antibody (Lyophilized)	2 vials
Human AIF Lyophilized Recombinant Protein	2 vials
Plate Seals	1 unit
Sample Diluent NS (ab193972)	1 x 12ml
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit
Stop Solution	1 x 12ml
TMB Development Solution	1 x 12ml

Function

Probable oxidoreductase that has a dual role in controlling cellular life and death; during apoptosis, it is translocated from the mitochondria to the nucleus to function as a proapoptotic factor in a caspase-independent pathway, while in normal mitochondria, it functions as an antiapoptotic factor via its oxidoreductase activity. The soluble form (AlFsol) found in the nucleus induces 'parthanatos' i.e., caspase-independent fragmentation of chromosomal DNA. Interacts with ElF3G,and thereby inhibits the ElF3 machinery and protein synthesis, and activates casapse-7 to amplify apoptosis. Plays a critical role in caspase-independent, pyknotic cell death in hydrogen peroxide-exposed cells. Binds to DNA in a sequence-independent manner.

Involvement in disease

Defects in AIFM1 are the cause of combined oxidative phosphorylation deficiency type 6 (COXPD6) [MIM:300816]. It is a mitochondrial disease resulting in a neurodegenerative disorder characterized by psychomotor delay, hypotonia, areflexia, muscle weakness and wasting.

Sequence similarities

Belongs to the FAD-dependent oxidoreductase family.

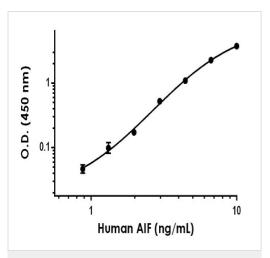
Post-translational modifications

Under normal conditions, a 54-residue N-terminal segment is first proteolytically removed during or just after translocation into the mitochondrial intermembrane space (IMS) by the mitochondrial processing peptidase (MPP) to form the inner-membrane-anchored mature form (AlFmit). During apoptosis, it is further proteolytically processed at amino-acid position 101 leading to the generation of the mature form, which is confined to the mitochondrial IMS in a soluble form (AlFsol). AlFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis in a caspase-independent manner.

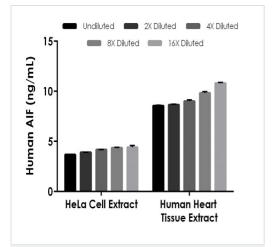
Cellular localization

Mitochondrion intermembrane space. Mitochondrion inner membrane. Cytoplasm. Nucleus. Cytoplasm > perinuclear region. Proteolytic cleavage during or just after translocation into the mitochondrial intermembrane space (IMS) results in the formation of an inner-membrane-anchored mature form (AIFmit). During apoptosis, further proteolytic processing leads to a mature form, which is confined to the mitochondrial IMS in a soluble form (AIFsol). AIFsol is released to the cytoplasm in response to specific death signals, and translocated to the nucleus, where it induces nuclear apoptosis. Colocalizes with EIF3G in the nucleus and perinuclear region.

Images



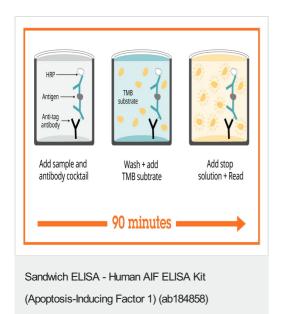
Example of human AIF standard curve in 1X Cell Extraction Buffer PTR.



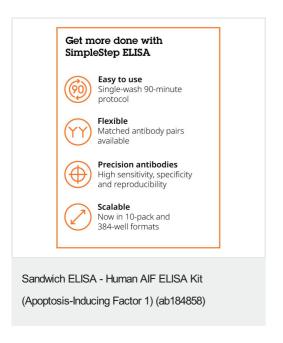
Interpolated concentrations of native AIF in human HeLa cell extract based on a 66.6 μ g/mL extract load and human heart tissue extract based on a 20 μ g/mL extract load.

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

The concentrations of AIF were measured in duplicate and interpolated from the AIF standard curve and corrected for sample dilution. The interpolated dilution factor corrected values are plotted (mean +/- SD, n=2). The mean AIF concentration was determined to be 4.106 ng/mL in HeLa cell extract and 9.364 ng/mL in human heart tissue extract.



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.



To learn more about the advantages of SimpleStep ELISA® kits see **here**.

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