Version: Datasheet-KE00772-A.0

Effective Date: 2025-05-28

Human ATG4D Sandwich ELISA Kit Datasheet

Please read it entirely before use

Catalogue Number: KE00772

Size: **96T**

Sensitivity: 0.06 ng/mL

Range: 0.39-25 ng/mL

Usage: For the quantitative detection of human ATG4D concentration in cell lysate.

This product is for research use only and not for use in human or animal therapeutic or diagnostic.

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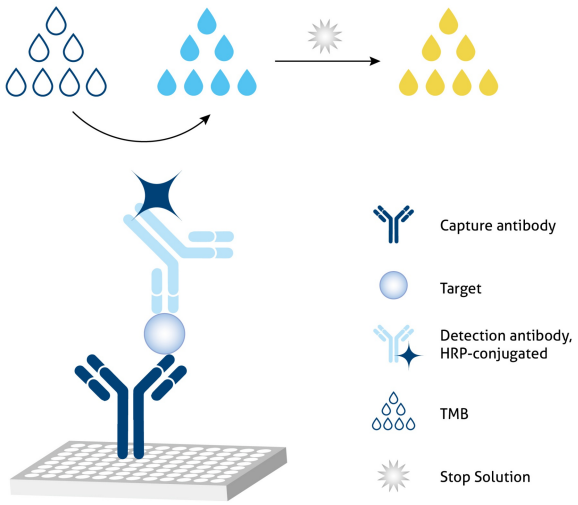
1. Background

ATG4D protein (Autophagy-related protein 4 homolog D) is a key protease in the autophagy regulatory pathway, belonging to the ATG4 protease family (including ATG4A, B, C, and D). Compared to other ATG4 family members (such as ATG4B), ATG4D exhibits tissue-specific expression, with particularly prominent activity in brain tissues, suggesting it may play unique functional roles in the nervous system. Studies have shown that ATG4D not only participates in classical autophagy but may also influence the progression of neurodegenerative diseases by regulating mitophagy and the clearance of abnormal protein aggregates.

2. Principle



Sandwich ELISA structure (Detection antibody labeled with HRP)

A capture antibody is pre-coated onto the bottom of wells which binds to analyte of interest. A detection antibody labeled with HRP also binds to the analyte. TMB acts as the HRP substrate and the solution color will change from colorless to blue. A stop solution containing sulfuric acid turns solution yellow. The color intensity is proportional to the quantity of bound protein which is measurable at 450 nm with the correction wavelength set at 630 nm.

3. Required Materials

3.1 A microplate reader capable of measuring absorbance at 450 nm with the correction wavelength set at 630 nm.

3.2 Calibrated, adjustable precision pipettes and disposable plastic tips. A manifold multi-channel pipette is recommended for large assays.

3.3 Plate washer: automated or manual.

3.4 Absorbent paper towels.

3.5 Glass or plastic tubes to prepare standard and sample dilutions.

3.6 Beakers and graduated cylinders.

3.7 Log-log or semi-log graph paper or computer and software for ELISA data analysis. A four-parameter logistic (4-PL) curve-fit is recommended.

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4. Kit Components and Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	**Unopened Kit:** Store at 2-8°C for 6 months or -20°C for 12 months. **Opened Kit:** All reagents stored at 2-8°C for 7 days. **Please use a new standard for each assay.**
Protein standard - 50 ng/bottle; lyophilized	2 bottles	
Detection antibody, HRP-conjugated (100×) - 120 uL/vial*	1 vial	
Sample Diluent PT 3-ef - 30 mL/bottle	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20×) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	4 pieces	

* Centrifugation immediately before use

5. Safety Notes

5.1 Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

5.2 Do not use the kit after the expiration date.

5.3 Do not mix or substitute reagents or materials from other kit lots or other sources.

5.4 Be sure to wear protective equipment such as gloves, masks and goggles during the experiment.

5.5 When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer to improve assay precision

6. Sample Collection and Storage

6.1 Cell Lysate:

1) Collect cells and wash by centrifuging at 500 x g for 5 minutes before resuspension in pre-cooled PBS buffer. Perform this step three times.

2) Count cells and then discard the supernatant.

3) Add protease inhibitor cocktail to the Extraction Reagent to a final concentration immediately prior to performing cell lysis.

4) Add 1 mL of Extraction reagent (containing protease inhibitor cocktail) Per 1 x 10^7 cells, Incubate cell suspension on ice for 30 minutes, use ultrasound to treat the samples.

5) Centrifuge cell lysate at 10,000 x g for 5 minutes at 4°C.

6) Measure the concentration of total protein in cell lysate using BCA assay. Where possible, keep samples on ice to avoid protein degradation.

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7. Reagent Preparation

7.1 Wash Buffer (1X): If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 30 mL of Wash Buffer Concentrate(20X) to 570 mL deionized or distilled water to prepare 1X Wash Buffer.

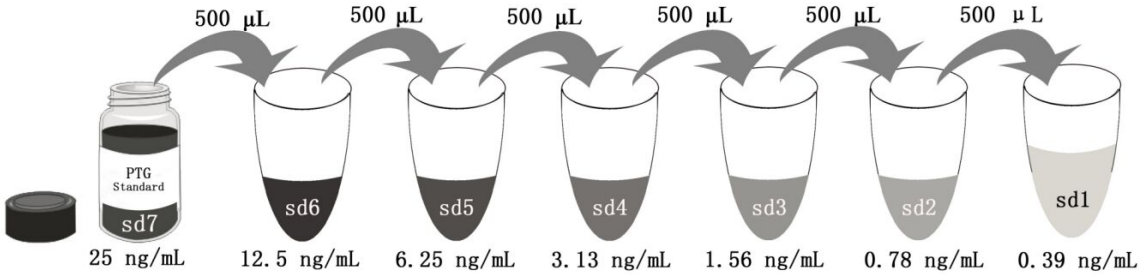
7.2 Detection Antibody, HRP-conjugated(1X): Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 uL 100X Detection Antibody, HRP-conjugated + 990 uL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated for a few seconds prior to use).

7.3 Sample Dilution: Different samples should be diluted with corresponding Sample Diluent, samples may require further dilution if the readout values are higher than the highest standard OD reading. Variations in sample collection, processing and storage may affect the results of the measurement.

Recommended Dilution for different sample types: 1:2 or 1:4 is recommended for cell lysate.

7.4 Standard Serial Dilution:

Add 2 mL Sample Diluent PT 3-ef in protein standard.



Add # μL of Standard diluted in the previous step	—	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
# μL of Sample Diluent PT 3-ef	**2000 μL**	500 μL	500 μL	500 μL	500 μL	500 μL	500 μL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

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8. Assay Procedure Summary

Bring all reagents to room temperature before use (Detection antibody, HRP-conjugated can be used immediately). To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

8.1 Take out the required number of microplate strips and return excess strips to the foil pouch containing the drying reagent pack and reseal; store at 4°C immediately. Microplate strips should be used in one week.

8.2 Preset the layout of the microplate, including control group,standard group and sample group, add 100 µL of each standard and sample to the appropriate wells.(Make sure sample addition is uninterrupted and completed within 5 to 10 minutes, It is recommended to assay all standards, controls, and samples in duplicate).

8.3 Seal plate with cover seal, pressing it firmly onto top of microwells. Incubate the plate for 2 hours at 37°C.

8.4 Wash

1) Gently remove the cover seal. Discard the liquid from wells by aspirating or decanting. Remove any residual solution by tapping the plate a few times on fresh paper towels.

2) Wash 4 times with 1X Wash Buffer, using at least 350-400 µL per well. Following the last wash, firmly tap plates on fresh towels 10 times to remove residual Wash Buffer. Avoid getting any towel fibers in the wells or wells drying out completely.

8.5 Add 100 µL of 1X Detection antibody, HRP-conjugated solution (refer to Reagent Preparation7.2) to each well. Seal plate with cover seal and incubate for 40 minutes at 37°C.

8.6 Repeat wash step in 8.4.

8.7 Signal development: Add 100 µL of TMB substrate solution to each well, protected from light. Incubate for 15 to 20 minutes. Substrate Solution should remain colorless until added to the plate.

8.8 Quenching color development: Add 100 µL of Stop Solution to each well in the same order as addition of the TMB substrate. Mix by tapping the side of the plate gently. NB: Avoid skin and eye contact with the Stop solution.

8.9 Read results: Immediately after adding Stop solution read the absorbance on a microplate reader at a wavelength of 450 nm. If possible, perform a double wavelength readout (450 nm and 630 nm).

8.10 Data analysis: Calculate the average of the duplicate readings (OD value) for each standard and sample, and subtract the average of the zero standard absorbance. Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis, use four-parameter logistic curve- fit (4-PL) analysis to do this. If the samples have been diluted, the OD readout from the standard curve must be multiplied by the dilution factor used.

Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 uL	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Detection antibody, HRP-conjugated Solution	100 uL	40 min	4 times	Cover Wells incubate at 37°C
3	TMB Substrate	100 uL	15-20 min	Do not wash	Incubate in the dark at 37°C
4	Stop Solution	100 µL	0 min	Do not wash	-
5	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

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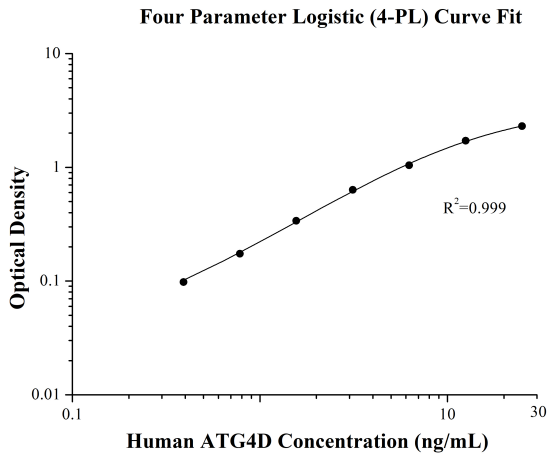
9. Validation Data

9.1 Standard curve

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.0947 0.0901	0.092	-
0.39	0.2053 0.1754	0.19	0.098
0.78	0.2707 0.2618	0.266	0.174
1.56	0.4373 0.4261	0.432	0.339
3.13	0.7319 0.7231	0.728	0.635
6.25	1.1593 1.1102	1.135	1.042
12.5	1.7775 1.8297	1.804	1.711
25	2.4102 2.3783	2.394	2.302

9.2 Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 8 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 16 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	8	21.04	0.52	2.49
2	8	6.22	0.35	5.59
3	8	2.83	0.15	5.29

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	16	21.74	1.16	5.32
2	16	6.02	0.34	5.68
3	16	2.88	0.18	6.31

9.3 Recovery

The recovery of human ATG4D spiked to three different levels throughout the range of the assay in cell lysate was evaluated.

Sample Type		Average% of Expected	Range (%)
Cell lysate	1:8	103	93-108

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9.4 Sample values

Cell lysate

	Human ATG4D (ng/mL)	Total protein (**mg/mL**)
SW 1990 cell lysate	18.18	2.20
A549 cell lysate	38.62	1.30
HeLa cell lysate	30.60	1.30
SH-SY5Y cell lysate	38.80	2.60

9.5 Sensitivity

The minimum detectable dose of human ATG4D is 0.06 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

9.6 Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Cell lysate
1:2	Average% of Expected	100
	Range (%)	-
1:4	Average% of Expected	79
	Range (%)	76-81
1:8	Average% of Expected	78
	Range (%)	76-80
1:16	Average% of Expected	88
	Range (%)	83-94

9.7 Specificity

This kit specifically recognizes native and recombinant human ATG4D.

10. References

1. Kauffman, Karlina J et al. Autophagy vol. 14,6 (2018): 992-1010.
2. Li, Min et al. The Journal of biological chemistry vol. 286,9 (2011): 7327-38.
3. Nguyen, Thanh Ngoc et al. Molecular cell vol. 81,9 (2021): 2013-2030.e9.
4. Tamargo-Gómez, Isaac et al. Cell death and differentiation vol. 28,9 (2021): 2651-2672.

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