Version: Datasheet-KE00873-A.0

Effective Date: 2025-07-07

# Human CEBP Alpha/CEBPA Sandwich ELISA Kit Datasheet

Please read it entirely before use

**Catalogue Number:** KE00873

**Size:** **96T**

**Sensitivity:** 0.02 ng/mL

**Range:** 0.625-40 ng/mL

**Usage:** For the quantitative detection of human CEBP Alpha/CEBPA 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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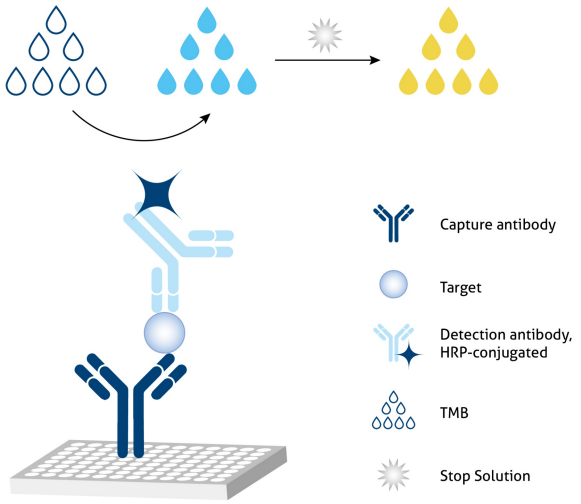
## 1. Background

CEBPA and its isoforms play important roles in lineage determination and gene activation in a variety of cell types by activating transcription from lineage-specific promoters. CEBPA is a DNA-binding protein that recognizes two different motifs: the CCAAT homology common to many promoters and the enhanced core homology common to many enhancers. In hematopoiesis, C/EBPa is a key factor in driving the development of myeloid cells interacting with a variety of factors, including c-Myc, PU.1, and microRNAs. It can also form heterodimers with the related proteins CEBP-beta and CEBP-gamma. The encoded protein has been shown to bind to the promoter and modulate the expression of the gene encoding leptin which plays an important role in body weight homeostasis. CEBPA can interact with CDK2 and CDK4, thereby inhibiting these kinases and causing growth arrest in cultured cells. Several pathways have been implicated as the means by which CEBPA mediates cell cycle arrest and proliferation, including p21, cyclin-dependent kinases and the E2F complex via c-Myc.

## 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** - 80 ng/bottle; lyophilized | 2 bottles | |
| **Detection antibody, HRP-conjugated (100×)** - 120 uL/vial* | 1 vial | |
| **Sample Diluent PT 4B1** - 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.

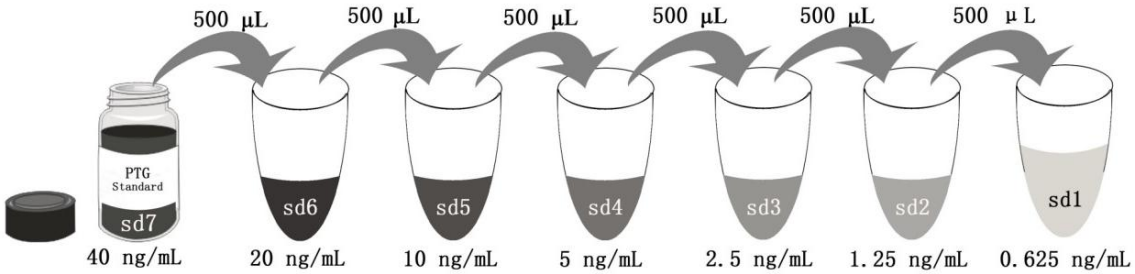
**7.2 Detection Antibody, HRP-conjugated(1X):** Dilute 100X Detection Antibody, HRP-conjugated 1:100 using Detection Diluent prior to assay. Suggested 1:100 dilution: 10 uL 100X Detection Antibody, HRP-conjugated + 990 uL Detection Diluent (Centrifuge the 100X Detection Antibody solution, HRP-conjugated 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 4B1 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 4B1 | **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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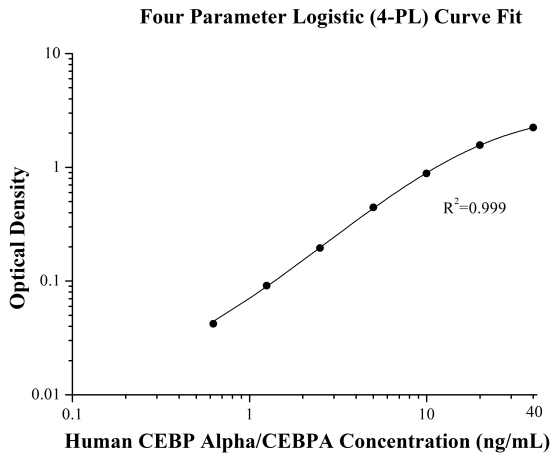
# 9. Validation Data

## 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 | 0.034<br>0.035 | 0.034 | - |
| 0.625 | 0.076<br>0.078 | 0.077 | 0.042 |
| 1.25 | 0.127<br>0.125 | 0.126 | 0.091 |
| 2.5 | 0.233<br>0.225 | 0.229 | 0.195 |
| 5 | 0.49<br>0.469 | 0.479 | 0.445 |
| 10 | 0.913<br>0.923 | 0.918 | 0.884 |
| 20 | 1.625<br>1.581 | 1.603 | 1.569 |
| 40 | 2.298<br>2.242 | 2.27 | 2.236 |

## 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 | 21.20 | 1.04 | 4.92 |
| 2 | 8 | 5.51 | 0.27 | 4.92 |
| 3 | 8 | 2.85 | 0.15 | 5.36 |

| Inter-assay Precision | | | | |
|---|---|---|---|---|
| Sample | n | Mean (ng/mL) | SD | CV% |
| 1 | 16 | 21.87 | 1.15 | 5.27 |
| 2 | 16 | 5.40 | 0.22 | 4.13 |
| 3 | 16 | 2.97 | 0.17 | 5.75 |

## 9.3 Recovery

The recovery of human CEBP Alpha/CEBPA spiked to three different levels throughout the range of the assay in cell lysate was evaluated.

| Sample Type | | Average% of Expected | Range (%) |
|---|---|---|---|
| Cell lysate | 1:16 | 82 | 71-88 |



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## 9.4 Sample values

### Cell lysate

| | Human CEBP Alpha/CEBPA (ng/mL) | Total protein (**mg/mL**) |
|---|---|---|
| THP-1 cell lysate | 39.15 | 2.90 |

## 9.5 Sensitivity

The minimum detectable dose of human CEBP Alpha/CEBPA is 0.02 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 | 86 |
| | Range (%) | 79-93 |
| 1:8 | Average% of Expected | 79 |
| | Range (%) | 74-85 |
| 1:16 | Average% of Expected | 84 |
| | Range (%) | 83-84 |

## 9.7 Specificity

This kit specifically recognizes native and recombinant human CEBP Alpha/CEBPA.

## 10. References


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5. Hall, Jonathan R et al. Cell cycle (Georgetown, Tex.) vol. 13,22 (2014): 3602-10.