

QuantiFluo™ Angiotensin-Converting Enzyme 1 Assay Kit (QACE1-100)

Quantitative Fluorometric Assay for ACE-1 Activity

DESCRIPTION

ANGIOTENSIN-CONVERTING ENZYME 1, ACE-1 (EC 3.4.15.1) is a critical enzyme in the renin-angiotensin-aldosterone system (RAAS), which regulates blood pressure, fluid, and electrolyte balance in the body. Found primarily in the lung endothelium and kidney epithelial cells, ACE-1 hydrolyzes angiotensin I, cleaving off the His-Leu dipeptide to produce angiotensin II. Its role in the RAAS system makes it a major target for hypertension and cardiovascular treatments.

BioAssay Systems' QACE1-100 Kit provides a convenient fluorometric method to measure ACE-1 activity in biological samples. In this assay, ACE-1 hydrolyzes the synthetic substrate, releasing the product. Then, o-phthalaldehyde (OPA) fluorescently labels the product for quantification. The increase in fluorescence at $\lambda_{ex/em} = 360/485$ nm is directly proportional to enzyme activity.

KEY FEATURES

Safe and convenient. Non-radioactive assay. "Mix-incubate-measure" type assay. No wash and reagent transfer steps are involved.

Sensitive and accurate. Linear detection range 0.01 - 5 U/L ACE-1 in a 96-well plate assay.

High-throughput. Can be readily automated to assay thousands of samples per day.

APPLICATIONS

For quantitative determination of ACE-1 activity determination in biological samples.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	5 mL	Standard:	100 µL
NaOH:	6 mL	Substrate:	50 µL
OPA:	100 µL		

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to the Material Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, the addition of the Working Reagent (WR) to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Reagent Preparation: Prior to the assay, equilibrate all components to room temperature and briefly centrifuge tubes before opening. The WR should be prepared fresh for each assay run.

Sample Preparation:

Serum: Should be diluted 5x or more in dH2O prior to the assay run.

Plasma: Incompatible with the assay, likely from interference with chelators due to the structure of ACE-1 as a metalloprotease.

Cell or tissue lysate: Can be assayed directly.

Standard Preparation: Vortex the Standard solution before each preparation. Precipitate formation is common but always redissolves and does not affect the assay. Prepare a 150 µM Premix by combining 10 µL Standard and 190 µL dH2O. Prepare standard dilutions and add 10 µL to the wells as follows.

No.	150 µM Premix + dH2O	Total Volume (µL)	Std (µM)
1	100 µL + 0 µL	100	150
2	60 µL + 40 µL	100	90
3	30 µL + 70 µL	100	45
4	0 µL + 100 µL	100	0

Reaction Preparation:

- Transfer 10 µL of each sample to separate wells of the plate for analysis. Also transfer 10 µL of each sample as a sample blank.
- Prepare enough WR for all wells by mixing 0.5 µL of Substrate and 45 µL of Assay Buffer for each well.
- Add 40 µL WR to all sample and standard wells. Tap plate to mix well and incubate at room temperature for 30 minutes. Set aside the remaining WR for addition to sample blank in step 5.
- During the 30-minute incubation, prepare Detection Reagent (DR) by mixing 1 µL OPA and 60 µL NaOH for each well.
- When the incubation is finished, add 40 µL of the remaining WR to the sample blank wells. Directly after, add 50 µL of DR to all wells. Incubate for 15 minutes at room temperature.
- Read fluorescence at $\lambda_{ex/em} = 360/485$ nm.

CALCULATION

Subtract the blank value (Standard #4) from the standard values and plot ΔF against the standard concentrations. Determine the slope (μM^{-1}) and calculate the ACE-1 activity in each sample as follows:

$$ACE-1 = \frac{F_{SAMPLE} - F_{SAMPLE BLANK}}{t \times \text{Slope } (\mu M^{-1})} \times n \text{ (U/L)}$$

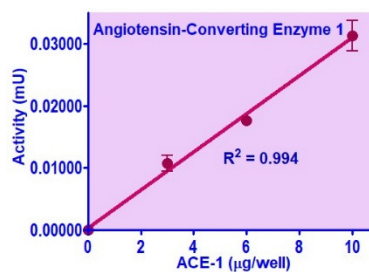
Where F_{SAMPLE} and $F_{SAMPLEBLANK}$ are the measured fluorescence values of the sample and sample blank, t is the reaction time (30 min), and n is the sample dilution factor.

Unit definition: 1 Unit (U) of ACE-1 will catalyze the conversion of 1 µmole of the Substrate per min at room temperature and pH 8.3.

Note: If sample ACE-1 activity exceeds 5 U/L, dilute samples in dH2O and repeat the assay.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), black, flat-bottom 96-well plates (e.g. Corning Costar), centrifuge tubes and a plate reader.



96-well Fluorometric ACE-1 Assay

Examples: A commercially available ACE-1 was assayed. Its activity was determined to be 3.06 ± 0.11 U/g. Duplicate assays for human serum ($n=5$), rat serum ($n=5$), and spiked and direct HEPG2 cell lysate ($n=1$) yielded ACE-1 activity of 0.90 ± 0.01 , 4.6 ± 0.1 , 1.2 ± 0.1 , and 0.10 ± 0.01 U/L, respectively. These values are examples and not intended as expected values.

LITERATURE

- Benson, J.R; Hare, P.E (1975). O-phthalaldehyde: fluorogenic detection of primary amines in the picomole range. Comparison with fluorescamine and ninhydrin: *Proc. Natl. Acad. Sci. U.S.A.* 72 (2) 619-622.
- Piepho, RW (2000). Overview of the angiotensin-converting-enzyme inhibitors. *Am J Health Syst Pharm.* 57 (1) S3-S7.
- Wong, M (2015). Angiotensin Converting Enzymes: *Handbook of Hormones.* 263–e29D-4.

