Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Renin Activity Fluorometric Assay Kit

rev. 6/18

(Catalog # K800-100; 100 assays; Store at -80°C)

I. Introduction:

Renin (EC 3.4.23.15), also known as an angiotensinogenase, is an enzyme that participates in the renin-angiotensin system (RAS) which mediates extracellular volume (i.e. blood plasma, lymph and interstitial fluid), and arterial vasoconstriction. An over-active renin-angiotensin system leads to vasoconstriction and retention of sodium and water, causing hypertension. Renin inhibitors are widely used for the treatment of hypertension. In BioVision's Renin Activity Assay Kit, Renin and other proteases hydrolyze the FRET substrate resulting in a product that is detected fluorometrically at Ex/Em = 328/552 nm to give total protease activity. In the presence of a Renin-Specific Inhibitor, hydrolysis of the substrate is only due to the non-specific protease activity. The difference between the total activity and the activity in the presence of Renin Specific Inhibitor, gives the Renin Activity in the sample. This rapid, simple & sensitive kit can detect renin activity as low as 0.75 U/ml.

II. Application:

- Detects Mammalian Renin Activity in samples containing renin.
- This kit is not intended to measure rat renin samples. For rat samples, see K806

III. Sample Type:

- Recombinant purified protein.

IV. Kit Contents:

Components	K800-100	Cap Code	Part Number
Renin Assay Buffer	25 ml	WM	K800-100-1
Renin Substrate	200 µl	Red	K800-100-2
Renin-Specific Inhibitor	20 µl	Blue	K800-100-3
Human Renin (Positive Control) (Lyophilized)	1 vial	Green	K800-100-4
EDANS Standard (100 µM)	100 µl	Yellow	K800-100-5

V. User Supplied Reagents and Equipments:

- 96-well white plate with flat bottom
- Fluorescence microplate reader

VI. Storage and Handling:

Store kit at -80°C, protected from light. Avoid repeated freeze/thaw for all non-buffer components. Briefly centrifuge small vials prior to opening. Read the entire protocol before using the kit.

VII. Reagent Preparation and Storage Conditions:

- **Human Renin (Positive Control):** Dissolve the lyophilized renin in 22 µl Renin Assay Buffer just before use. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use.

VIII. Renin Activity Assay protocol:

1. **Sample Preparation:** Add 2-48 µl samples into each well and adjust the volume to 50 µl with Renin Assay Buffer. For Background Control (non-specific protease activity), dilute Renin-Specific Inhibitor 10 times by adding 1 µl Renin-Specific Inhibitor to 9 µl Renin Assay Buffer just before use. Add the same amount of samples as used for checking the Renin Activity into desired well(s) and add 2 µl diluted Renin Specific Inhibitor. Adjust the volume to 50 µl with Renin Assay Buffer.
2. **EDANS Standard:** Dilute EDANS Standard to 10 µM by adding 10 µl of 100 µM EDANS Standard to 90 µl Renin Assay Buffer. Add 0, 2, 4, 6, 8, 10 µl of diluted 10 µM EDANS Standard into a series of wells to generate 0, 20, 40, 60, 80 and 100 pmol/well EDANS Standard. Adjust the volume to 100 µl with Renin Assay Buffer.
   Note:
   Dilute the EDANS Standard just before use & discard any unused Standard.
3. **Positive Control:** Add 2-6 µl of Human Renin into desired well(s) and adjust the volume to 50 µl with Renin Assay Buffer.
4. **Reaction Mix:** Make enough reagents for the number of assays to be performed. For each well, prepare 50 µl mix containing:

	Reaction Mix
Renin Assay Buffer	48 µl
Renin Substrate	2 µl

   Add 50 µl of Reaction Mix to each well containing the Positive Control, Samples and Background Controls. Mix well.
5. **Measurement:** Measure the fluorescence (Ex/Em = 328/552 nm) in kinetic mode for 30-60 min. at 37°C. Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding RFU for Sample (R_{S1} and R_{S2}) and Background (R_{B1} and R_{B2}). The EDANS Standard Curve can be read in endpoint mode (i.e., at the end of incubation time).
6. **Calculations:** Plot the EDANS Standard Curve. Calculate the renin activity of the test sample $\Delta RFU = (R_{S2} - R_{S1}) - (R_{B2} - R_{B1})$. Apply the ΔRFU to the Standard Curve to get B pmoles of EDANS liberated during the reaction time ($\Delta T = T_2 - T_1$).

$$\textbf{Sample Renin Activity} = \frac{B}{\Delta T \times V} \times \textbf{Dilution Factor} = \textbf{pmol/min/ml} = \textbf{mU/ml}$$

Where: **B** is the EDANS amount from the Standard Curve (pmol).
Δ**T** is the reaction time (min.)
V is the sample volume added into the reaction well (ml).

Sample Renin Activity can also be expressed as mU/mg of protein.

Unit Definition: One unit of Renin is the amount of enzyme that hydrolyzes the substrate to yield 1.0 nmol of EDANS per min. at 37°C.

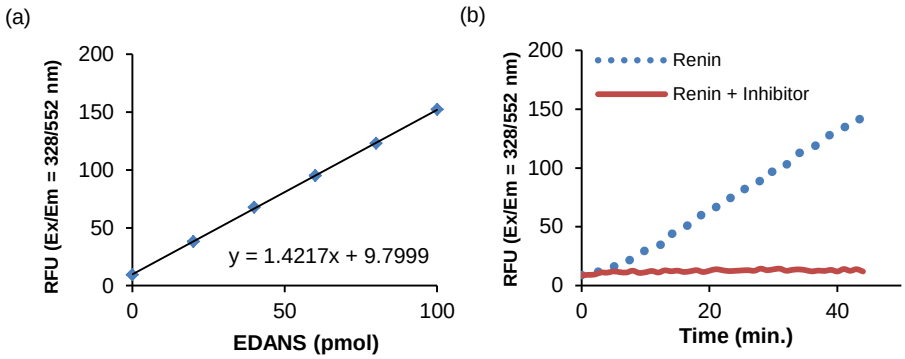

Figure: (a) EDANS Standard Curve (b) Renin Activity in Positive Control. Assays were performed following kit protocol.

IX. RELATED PRODUCTS:

Renin Inhibitor Screening Kit (K799)
Renin, His-Tagged, Human Recombinant (6300)
ACE2 (Human) ELISA Kit (K4918)
Renin Inhibitor (2084)
Angiotensin II, Human (4917)
Valsartan (2004)

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