Renin Fluorimetric Assay Kit

Catalog No. KM0136

Detection and Quantification of Renin in Biological Samples.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.
INTRODUCTION

Renin, also known as angiotensinogenase, is an enzyme that mediates extracellular volume and arterial vasoconstriction. It regulates the body’s mean arterial blood pressure and electrolyte homeostasis as the first and rate-limiting step of the renin-angiotensin system RAS cascade. As renin circulates in the bloodstream, it hydrolyzes angiotensinogen from the liver into angiotensin I, which is later converted to angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II is a vasoconstrictor and causes the heart to work more vigorously, resulting in hypertension. In addition, angiotensin II stimulates the adrenal glands to release aldosterone and ADH, which further contributes to heightened blood pressure in addition to raised blood volume. Since an overactive RAS leads to vasoconstriction and hypertension, renin inhibitors have been used to counter these symptoms.

This assay allows for high-throughput screening of renin inhibitors and continuous assay of renin activity by using proprietary fluorescence resonance energy transfer (FRET) peptide that reacts with renin to form a red fluorescent product. Signals increase with increasing renin activity, and can be monitored with fluorescence microplate reader with excitation at 540 nm and emission at 590 nm.

FEATURES

Number of Assays: 100 Assays
Fluorimetric Dynamic Range: Detect as little as 1 ng renin in 100 uL volume
Ex/Em = 540/590 nm
Non-radioactive
Continuous

STORAGE & HANDLING

Upon receipt, store all components in the original box at -20°C, protected from light. The kit should be stable for at least six months.

Note: Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
KIT CONTENTS & STOCK PREPARATION

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Microplate</td>
<td>1 plate</td>
<td>RT</td>
</tr>
<tr>
<td>Dye Reagent</td>
<td>1 vial</td>
<td>-20°C</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>10 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>Renin Standard</td>
<td>1 vial (40 ug/ml, 25 uL)</td>
<td>-20°C</td>
</tr>
<tr>
<td>DMSO</td>
<td>200 uL</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

Note: If not running 100 assays at once, aliquot and store reagents under conditions according to this table. Use within 2 months.

MATERIALS NOT PROVIDED

Pipetting devices, tubes, and microplate reader.

ASSAY RESTRICTIONS

- This kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in performance of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.
REAGENT & SAMPLE PREPARATIONS
Please read the manual in its entirety before proceeding with the assay.

Note: Warm all reagents to room temperature before use and protect from light. Aliquot and store the reagents at -20°C if not all of it is used.

1. Prepare Renin containing biological samples as desired.

2. Prepare 100X Dye Reagent stock solution by adding 50 μL of DMSO into the vial of Dye Reagent. 
   Note: The stock solution should be used promptly. Any remaining solution should be aliquoted and frozen at -20°C.

3. Prepare Renin Assay Mixture by dilute reconstituted 100X Dye Reagent stock solution (from Step 2) with Assay Buffer at 1:100 as shown in Table 1.

Table 1: Renin Assay Mixture for one 96-well plate (100 assays)

<table>
<thead>
<tr>
<th>Components</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 X Dye Reagent stock Solution (from Step 2)</td>
<td>50 μL</td>
</tr>
<tr>
<td>Assay Buffer</td>
<td>5 mL</td>
</tr>
</tbody>
</table>

4. Prepare serially diluted Renin standards (0 to 1 μg/mL)

4.1 Add 12.5 μL of 40 μg /mL Renin Standard into 487.5 μL of Assay Buffer to get 1 μg /mL Renin standard solution.

4.2 Take 150 μL of 1 μg/mL Renin standard solution (from Step 4.1) to perform 1:3 serial dilutions to get 300, 100, 30, 10, 3, 1 and 0 ng /mL serially diluted Renin standards.

4.3 Add Renin standards and/or Renin -containing test samples into a black wall/solid bottom 96-well microplate as described in Tables 2 and 3.

Table 2. Layout of Renin standards and test samples in a solid black 96-well microplate

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>BL</td>
<td>BL</td>
<td>TS</td>
<td>TS</td>
<td>...</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>B</strong></td>
<td>Ren 1</td>
<td>Ren 1</td>
<td>...</td>
<td>...</td>
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<tr>
<td><strong>C</strong></td>
<td>Ren 2</td>
<td>Ren 2</td>
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<tr>
<td><strong>D</strong></td>
<td>Ren 3</td>
<td>Ren 3</td>
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<tr>
<td><strong>E</strong></td>
<td>Ren 4</td>
<td>Ren 4</td>
<td></td>
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</tr>
<tr>
<td><strong>F</strong></td>
<td>Ren 5</td>
<td>Ren 5</td>
<td></td>
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<tr>
<td><strong>G</strong></td>
<td>Ren 6</td>
<td>Ren 6</td>
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</tr>
<tr>
<td><strong>H</strong></td>
<td>Ren 7</td>
<td>Ren 7</td>
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</tbody>
</table>

Note: Ren= Renin Standards, BL=Blank control, TS=test samples.

Table 3. Reagent composition for each well
<table>
<thead>
<tr>
<th>RENIN Standard</th>
<th>Blank Control</th>
<th>Test Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Dilutions*: 50 μl</td>
<td>Assay Buffer: 50 μl</td>
<td>50 μl</td>
</tr>
</tbody>
</table>

Note 1: Add the serially diluted Renin standards from 1 ng/mL to 1000 ng/mL into each well from Ren 1 to Ren 7 in duplicate.

Note 2: for 384-well plates, use 25 μL/well.
ASSAY PROTOCOL

5. Run the enzyme reaction

5.1 Pre-incubate the plate at a desired temperature for the enzyme reaction (e.g. 25°C or 37°C) for 10-15 min if you are screening Renin inhibitors.

5.2 Add 50 μL (96-well) or 25 μL (384-well) of Dye Reagent solution (from Step 3) to the sample and control wells of the assay plate.

5.3 Incubate the reaction at 37°C incubator for 30 to 60 minutes.

5.4 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 540/590 nm (cut off = 570 nm).

For kinetic reading: Immediately start measuring fluorescence intensity and continuously record data every 5 minutes for 30 to 60 minutes.

For end-point reading: Incubate the reaction at 37°C for 60 minutes or longer, kept from light if possible. And then measure the fluorescence intensity.
STANDARD CURVE

The fluorescence in the substrate control well is used as a control, and is subtracted from the values for other wells with the enzyme reactions.

Figure 1. Renin dose response was measured with Renin Assay Kit in a 96-well black solid plate. As low as 10 ng/mL Renin was detected with 60 minutes incubation in 37°C.
TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com or contact us at tech@immunoway.com

NOTES