### DESCRIPTION

E. coli -derived human Renalase protein

<table>
<thead>
<tr>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala2-Ile342</td>
</tr>
<tr>
<td>Accession # Q5VYX0-1</td>
</tr>
<tr>
<td>with substitution Glu37Asp, N-terminal Met and 6-His tag</td>
</tr>
</tbody>
</table>

### N-terminal Sequence Analysis

<table>
<thead>
<tr>
<th>Predicted Molecular Mass</th>
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<tbody>
<tr>
<td>39 kDa</td>
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</tbody>
</table>

### SPECIFICATIONS

#### SDS-PAGE

<table>
<thead>
<tr>
<th>Activity</th>
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<tbody>
<tr>
<td>Measured by its ability to reduce Resazurin. The specific activity is &gt;3.5 pmol/min/µg, as measured under the described conditions.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endotoxin Level</th>
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<tbody>
<tr>
<td>&lt;1.0 EU per 1 µg of the protein by the LAL method.</td>
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</table>

<table>
<thead>
<tr>
<th>Purity</th>
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<tbody>
<tr>
<td>&gt;95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.</td>
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<table>
<thead>
<tr>
<th>Formulation</th>
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<tr>
<td>Supplied as a 0.2 µm filtered solution in Tris, EDTA, DTT and Glycerol. See Certificate of Analysis for details.</td>
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</table>

### Activity Assay Protocol

#### Materials

- Assay Buffer: 50 mM HEPES, 200 mM NaCl, 5 µM FAD (Flavin Adenine Dinucleotide), 0.05% Tween-20, pH 7.5
- Recombinant Human Renalase (rhRenalase) (Catalog # 9887-RE)
- β-Nicotinamide adenine dinucleotide, reduced (NADH) (Sigma, Catalog # N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
- Resazurin (Catalog # AR002) (MW = 251.17 Da)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

#### Assay

1. Dilute rhRenalase to 40 µg/mL in Assay Buffer.
2. Prepare a Substrate Mixture containing 400 µM of NADH and 20 µM Resazurin in Assay Buffer. Mix well and use immediately.
3. Load 50 µL of 40 µg/mL rhRenalase into a plate, and start reaction by adding 50 µL Substrate Mixture. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL Substrate Mixture.
4. Read plate at excitation and emission wavelengths of 540 and 585 nm, respectively, in kinetic mode for five minutes.
5. Calculate specific activity:

\[
\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{\text{max}} \times \text{Conversion Factor}}{\text{amount of enzyme (µg)}}
\]

*Adjusted for Substrate Blank.
**Derived using calibration standard Resorufin (Sigma, Catalog # R3257).

### Final Assay Conditions

#### Per Well:

- rhRenalase: 2.0 µg
- NADH: 200 µM
- Resazurin: 10 µM

### PREPARATION AND STORAGE

#### Shipping

The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

#### Stability & Storage

Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

### DATA
Enzyme Activity
Recombinant Human Renalase (Catalog # 9887-RE) is measured by its ability to reduce Resazurin (Catalog # AR002).

BACKGROUND
Renalase has been reported to regulate cardiac function and blood pressure and initially proposed to metabolize circulating catecholamines (1). Recent characterization has clarified the true enzymatic function of renalase as oxidation of NAD(P)H isomers to B-NAD(P)+ (2, 3). As these molecules are potent inhibitors of primary metabolism dehydrogenases, cellular renalase activity serves to prevent the inhibitory effects of these molecules on primary metabolism (2, 3). Renalase was initially found to have robust expression in the kidney (1) and later confirmed to have systemic expression in all tissues. Renalase is classified as a member of the flavoprotein superfamily but uniquely lacks an internal structural element that causes its active site to be solvent exposed (4). Renalase contains a putative secretory N-terminal signal sequence (aa 1-17) that also contains residues required for FAD cofactor binding, suggesting that the enzyme would require the N-terminus to remain functionally active (3). However, renalase is found both intracellularly and secreted extracellularly. Extracellular renalase is cleaved during secretion from the cell (5). Circulating renalase acts as a cytokine, resulting in extensive reported physiological effects (6). Increases in blood pressure was induced by renalase gene knockout (7) and gene deletion aggravates acute ischemic kidney (8). Polymorphisms in renalase are associated with hypertension and stroke in type 2 diabetes (9, 10). Full-length recombinant renalase as well as truncated, non-active, peptides were found to promote cell and organ survival in a process independent of enzymatic activity via signaling through the receptor PMCA4b, characterized as an ATPase involved in cell signaling and cardiac hypertrophy (11, 12). Renalase was significantly increased in pancreatic, bladder, breast and melanoma cancers where its signaling role favors cell survival and tumor growth (13). Consequently, renalase inhibition has been suggested to provide a novel therapeutic option for cancer treatment (13).

References: