**DESCRIPTION**

**Source**
Spodoptera frugiperda, Sf 21 (baculovirus)-derived

<table>
<thead>
<tr>
<th>Human Calcineurin A</th>
<th>Human Calcineurin B</th>
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</thead>
<tbody>
<tr>
<td>(Ser2-Gln521)</td>
<td>(Met1-Val170)</td>
</tr>
<tr>
<td>Accession # NP_000935</td>
<td>Accession # NP_000936</td>
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</tbody>
</table>

**N-terminal Sequence**
Asp15 (Calcineurin A) & Asp17 (Calcineurin B)

**Predicted Molecular Mass**
57 kDa (Calcineurin A), 18 kDa (Calcineurin B)

**SPECIFICATIONS**

**SDS-PAGE**
43-65 kDa & 15-20 kDa, reducing conditions

**Activity**
Measured by its ability to dephosphorylate the peptide substrate, DLDVPIPGRFDRRVS(PO₃)₅VAAE (Catalog # ES012). The specific activity is >900 nmol/min/mg, under the described conditions.

**Endotoxin Level**
<1.0 EU per 1 μg of the protein by the LAL method.

**Purity**
>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.

**Formulation**
Supplied as a 0.2 μm filtered solution in HEPES, CaCl₂, MgCl₂, DTT and Glycerol. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Assay Buffer: 20 mM Tris, 10 mM MgCl₂, 0.1 mM CaCl₂, 1 mg/mL BSA, pH 7.5
- Recombinant Human Calcineurin (rhCalcineurin) (Catalog # 3160-CA)
- Substrate: Serine/Threonine Phosphatase substrate I (Asp-Leu-Asp-Val-Pro-Ile-Pro-Gly-Arg-Asp-Arg-Val-Ser(PO₃)₂-Val-Ala-Ala-Glu) (Catalog # ES012), MW: 2192 Da. Prepare a 1 mM stock in diH₂O
- Calmodulin (MW: 16.8 kDa) (Sigma, Catalog # P2277). Prepare a 10 μM stock in Assay Buffer
- EDTA (Sigma, Catalog # E6758), prepare a 0.1 M stock in deionized water (pH 8.0)
- Incubator at 37 °C able to shake samples
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Sealers (Corning, Catalog # 3095)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**
1. Dilute rhCalcineurin to a concentration of 0.71 μg/mL in Assay Buffer. Add 35 μL to each well.
2. Dilute Calmodulin to a concentration of 16.8 μg/mL (1 μM) in Assay Buffer. Add 5 μL to each well. As a control add 5 μL EDTA instead of Calmodulin to fully inhibit rhCalcineurin.
3. Cover plate, tap to mix and incubate plate at room temperature for 1 hour.
4. After incubation remove plate cover and add 10 μL of 1 mM Substrate.
5. Place cover back over plate and incubate at 37 °C with shaking for 30 min.
6. During second incubation, prepare phosphate standard curve. Prepare serial dilutions in Assay Buffer at the following concentrations: 100, 50, 25, 12.5, 6.25, 3.13, and 1.57 μM.
7. At the end of the incubation, quickly add 50 μL of the phosphate curve to the plate, in duplicate, including a 0 μM phosphate point (50 μL Assay Buffer).
8. Add 10 μL of Malachite Green Reagent A, tap to mix, and incubate at room temperature for 10 minutes.
9. Add 10 μL of Malachite Green Reagent B, tap to mix, and incubate at room temperature for 20 minutes.
10. Read at 620 nm and determine phosphate liberated from samples using standard curve.
11. Calculate the specific activity:

   \[ \text{Specific Activity (nmol/min/mg)} = \frac{\text{Phosphate released} \times \text{Incubation time (min)} \times \text{amount of enzyme (mg)}} {\text{Incubation time (min)} \times \text{amount of enzyme (mg)}} \]

   *Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Substrate Blank.

**Final Assay Conditions**
- Per Well:
  - Phosphate standard curve: 5.0, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0 nmol
  - Calcineurin: 0.00002485 mg

**PREPARATION AND STORAGE**

**Shipping**
The product is shipped with dry ice or equivalent. 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.
Calcineurin, also called Protein Phosphatase 2B, PP2B, Protein Phosphatase 3, and PPP3, is an enzyme that dephosphorylates serine and threonine residues in proteins. It is a heterodimer of a 59,000 dalton catalytic A subunit and a 19,000 dalton regulatory B subunit that is activated by the binding of calcium ions and calmodulin (1). Calcineurin is expressed in many tissues, but its levels are highest in the brain, where it may play a role in learning and memory (2). It has many substrates, including NFAT, a transcription factor that is activated by dephosphorylation (3). Complexes of the immunosuppressants cyclosporin and FK506 with immunophilin proteins such as cyclophilin and FKBP12 are potent and specific inhibitors of Calcineurin activity (4). Alterations in Calcineurin activity are suspected to play a role in cardiac hypertrophy (5) and graft versus host disease in organ transplantation (6).

References: