Recombinant Human Cyclophilin A

Catalog Number: 3589-CA

DESCRIPTION

Source	*E. coli*-derived human Cyclophilin A protein Met1-Glu165 Accession # P62937
N-terminal Sequence Analysis	Met1
Predicted Molecular Mass	18 kDa

SPECIFICATIONS

SDS-PAGE	17 kDa, reducing conditions
Activity	Measured by its ability to inhibit calcineurin phosphatase activity in the presence of Cyclosporin A. The IC_{50} for inhibition of calcineurin activity is <300 nM, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer (AB): 20 mM Tris, 10 mM $MgCl_2$, 0.1 mM $CaCl_2$, 1 mg/mL BSA, pH 7.5
- Recombinant Human Cyclophilin A (rhCyclophilin A) (MW: 18 kDa) (Catalog # 3589-CA)
- Recombinant Human Calcineurin (rhCalcineurin) (Catalog # 3160-CA)
- Substrate: Serine/Threonine Phosphatase Substrate I (Catalog # ES012), 1 mM in diH_2O
- Calmodulin (MW: 16.8 kDa) (Sigma, Catalog # P2277), 10 µM in Assay Buffer
- EDTA (Sigma, Catalog # E6758), 0.1 M in diH_2O (pH to 8.0)
- Cyclosporin A (Sigma, Catalog # C1832), 1 mM in DMSO
- Incubator at 37 °C able to shake microplate
- 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 Calcineurin to 1.17 µg/mL, Calmodulin to 16.8 µg/mL (1 µM), and Cyclosporin A to 30 µM in AB.
2. Dilute Cyclophilin A in AB to the following pre-incubation conc.: neat, 10000, 5000, 2500, 1250, 625, 313, 156, 78, 39 and 20 nM.
3. Add the following to the wells of a plate (add largest volume last to mix all together):

Samples	Pos. Control (prepare two)	Neg. Control (optional, prepare one)
5 µL of Cyclophilin A at pre-incubation conc.	5 µL of assay buffer	5 µL of EDTA
5 µL of Calmodulin	5 µL of Calmodulin	5 µL of Calmodulin
5 µL of Cyclosporin A	5 µL of Cyclosporin A	5 µL of Cyclosporin A
25 µL of Calcineurin	25 µL of Calcineurin	25 µL of Calcineurin

4. Seal plate, tap to mix and incubate plate at room temp for 1 hr.
5. After incubation remove seal and add 10 µL of Substrate.
6. Reseal and incubate at 37 °C with shaking for 1 hr.
7. During second incubation, prepare kit standard curve. Prepare serial dilutions in AB at the following conc.: 100, 50, 25, 12.5, 6.25, 3.13, and 1.57 µM.
8. Add 50 µL of the phos. curve to the plate, in duplicate, including a 0 mM phos. point (50 µL AB).
9. To all wells add 10 µL of kit reagent A, tap to mix, and incubate at RT for 10 min.
10. To all wells add 10 µL of kit reagent B, tap to mix, and incubate at RT for 20 min.
11. Read at 620 nm.
12. Determine the 50% inhibiting conc. (IC_{50}) of rhCyclophilin A by plotting conc. (nM) vs. Phos. released (nmol) with 4-PL fitting.
13. The specific activity of rhCalcineurin at each point can be determined using the following eqn. (if needed):

$$\text{Specific Activity (nmol/min/mg)} = \frac{\text{Phosphate released* (nmol)}}{\text{Inc. time (min) x amount of enzyme (mg)}}$$

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

Final Assay Conditions

Per Well:

- Phos. standard: 5.0, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, and 0 nmoles
- rhCyclophilin A: nt/14, 714, 357, 179, 89, 45, 22, 11, 5.6, 2.8, 1.4, 0 nM
- rhCalcineurin: 0.00002925 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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Human Cyclophilin A

Catalog Number: 3589-CA

BACKGROUND

Cyclophilin A, also called Peptidyl-prolyl Isomerase A, PPIA, CYPA, and CYPH, was originally characterized for its ability to catalyze the transition between cis- and trans- proline residues critical for proper folding of proteins (1). Cyclophilin is also incorporated into many viruses, including HIV-1, where it has been speculated to be involved in functions such as viral assembly and infectivity (2). The immunosuppressive activity of cyclosporins has been correlated with their ability to form complexes with cyclophilins that inhibit calcineurin phosphatase activity (3) and prevent incorporation of cyclophilin into viral particles (4). The cyclosporin/cyclophilin complex selectively binds and inactivates calcineurin (3, 5), making it a useful inhibitor for studying calcineurin activity.

References:

1. Hamilton, G.S. and J.P. Steiner (1998) J. Med. Chem. **41**:5119.
2. Cantin, R. *et al.* (2005) J. Virology **79**:6577.
3. Liu, J. *et al.* (1992) Biochemistry **31**:3896.
4. Wiegers K. and H.G. Krausslich (2002) Virology **294**:289.
5. Liu, J. *et al.* (1991) Cell **66**:807.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449