Recombinant Mouse Testican 3/SPOCK3

Catalog Number: 2346-PI

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Ala26-Ile436, with a C-terminal 6-His tag Accession # Q8BKV0
N-terminal Sequence Analysis	Ala26
Predicted Molecular Mass	47 kDa

SPECIFICATIONS

SDS-PAGE	58-70 kDa, reducing conditions
Activity	Measured by its ability to inhibit active Cathepsin L cleavage of a fluorogenic peptide substrate Z-LR-AMC (Catalog # ES008). The IC_{50} value is approximately 15 nM, under the described conditions. See Activity Assay Protocol on www.RnDSystems.com
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 5 mM DTT, pH 6.0
- Recombinant Mouse Testican 3/SPOCK3 (rmTestican 3) (Catalog # 2346-PI)
- Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-CY)
- Substrate: Z-Leu-Arg-AMC (Catalog # ES008), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute rhCathepsin L to 40 µg/mL in Assay Buffer.
2. Incubate diluted rhCathepsin L on ice for 15 minutes (to activate).
3. Prepare a curve of rmTestican 3 (MW: 47,725 Da) in Assay Buffer. Make the following serial dilutions: 1200, 600, 300, 200, 133.3, 88.9, 59.2, 39.5, 19.75, and 3.95 nM.
4. Dilute activated rhCathepsin L to 0.5 µg/mL in Assay Buffer.
5. Combine 25 µL of 0.5 µg/mL rhCathepsin L with 25 µL of rmTestican 3 serial curve dilutions. Include two controls containing 25 µL Assay Buffer with 25 µL of 0.5 µg/mL rhCathepsin L.
6. Incubate reaction mixtures at 37 °C for 15 minutes.
7. After incubation, dilute the reaction mixtures with 200 µL (1/5 dilution) of Assay Buffer.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. In a plate, load 50 µL of the diluted incubated mixtures, and start the reaction by adding 50 µL of 20 µM Substrate to wells.
10. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) of rmTestican 3 by plotting RFU/min (or specific activity) vs. concentration with 4-PL fitting.
12. The specific activity for rhCathepsin L at each point may be determined using the following formula (if needed):

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).

Final Assay Conditions

Per Well:

- rhCathepsin L: 0.0025 µg
- rmTestican 3 curve: 60, 30, 15, 10, 6.665, 4.445, 2.96, 1.975, 0.9875, and 0.1975 nM.
- Substrate: 10 µM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 100 µg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. 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 reconstitution.

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BACKGROUND

Testican 3 encoded by the SPOCK3 gene is a proteoglycan expressed in brain (1). The human and mouse cDNAs predict 90% identity between the deduced amino acid sequences from the two species, indicating a conserved function (2, 3). Only the human protein, but not the mouse protein, has been characterized in the literature. Testican 3 contains Ca^{2+}-binding domain and the C-terminal acidic domain with putative glycosaminoglycan attachment sites. In addition, it contains three potential inhibitory domains targeted toward three different classes of proteases, metallo, cysteine and serine proteases. The N-terminal region, which is unique to testicans, is responsible for the inhibition of Testican 3 towards MMP-14 (MT1-MMP, a metalloprotease) activation of MMP-2 (1). The thyropin domain and the follistatin-like domain with a six cysteine Kazal-like motif may inhibit cysteine and serine proteases, respectively (4). A spliced variant designated as N-Tes contains the N-terminal unique region, the follistatin-like domain and the Ca^{2+}-binding domain, but lacks the C-terminal thyropin domain and the acidic domain (1). The purified recombinant mouse (rm) Testican 3 is capable of inhibiting recombinant human (rh) MMP-14 and rhCathepsin L (Catalog # 918-MP and 952-CY) in assays using the fluorogenic peptide substrates (Catalog # ES001 and ES008). As compared to rhTestican 1 (Catalog # 2327-PI), the IC_{50} of rmTestican 3 is weaker toward rhCathepsin L and rhMMP-14 activity.

References:

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3. Okazaki, Y. *et al.* (2002) Nature **420**:563.
4. Alliel, P.M. *et al.* (1993) Eur. J. Biochem. **214**:347.

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