E-64

Catalog Number: EI004
Lot Number: TDB02

Specifications and Use

Product	♦ *trans*-Epoxysuccinyl-leucylamido-[4-guanidino]butane
Molecular Mass	♦ 357.41 Da
Purity	♦ >95% by high performance liquid chromatography
Quantity	♦ 10 mg
Effective Concentration	♦ 5 - 50 μM
Activity and Applications	♦ Measured by its ability to inhibit Recombinant Human Cathepsin L (Catalog # 952-CY). ♦ The IC_{50} is <5 nM, as measured under the described conditions. See Activity Assay Protocol on next page for details. ♦ E-64 is an irreversible inhibitor of papain-like cysteine proteases such as Cathepsin-L, bromelain, and calpains (1).
Formulation	♦ Supplied as a 50 mM solution in DMSO.
Dilution	♦ It is recommended that the first dilution be no less than 50 fold into an aqueous solution.
Storage	♦ Stable for 6 months after time of receipt when stored at -20° C to -80° C **in a manual defrost freezer.**

References:

1. Beynon, R. and J.S. Bond, 2001, *Proteolytic Enzymes: A Practical Approach*, Oxford University Press.

R&D Systems, Inc.
1-800-343-7475

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 5 mM DTT, pH 6.0
- E-64 (Catalog # EI004), 50 mM stock in DMSO
- Recombinant Human Cathepsin L (rhCathepsin L) (Catalog # 952-CY)
- Substrate: Z-Leu-Arg-AMC (Catalog # ES008), 2 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 on ice for 15 minutes.
3. After incubation, dilute activated rhCathepsin L to 0.2 µg/mL in Assay Buffer.
4. Prepare a curve of E-64 in Assay Buffer. Make the following serial dilutions: 1000000 nM, 20000 nM, 500 nM, 50 nM, 25 nM, 10 nM, 5 nM, 2 nM, 1 nM, and 0.1 nM.
5. Gently mix 30 µL of each of the E-64 curve dilutions with 30 µL of the 0.2 µg/mL rhCathepsin L. Include a control (in duplicate) containing 30 µL Assay Buffer and 30 µL of the 0.2 µg/mL rhCathepsin L.
6. Incubate mixtures at room temperature for 15 minutes.
7. Dilute reaction mixture by adding 90 µL of Assay Buffer to each reaction.
8. Dilute Substrate to 20 µM in Assay Buffer.
9. Load 50 µL of the incubated mixtures into a black well plate, and start the reaction by adding 50 µL of 20 µM Substrate.
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}) for E-64 by plotting RFU/min (or specific activity) versus 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 (Sigma, Catalog # A-9891)

Final Assay Conditions Per Well

rhCathepsin L (MW: 25,524): 0.002 µg (0.784 nM)
E-64 curve: 100000 nM, 2000 nM, 50 nM, 5 nM, 2.5 nM, 1 nM, 0.5 nM, 0.2 nM, 0.1 nM, and 0.01 nM
Substrate: 10 µM