

BACTERIAL TOXINS FOR RESEARCH AND INDUSTRY

PRODUCT INFORMATION

MAPKKide®

FOR FLUOROMETRIC MEASUREMENT OF LETHAL FACTOR (LF) ACTIVITY

Anthrax toxin is responsible for the symptoms associated with anthrax.¹ Three proteins are collectively known as anthrax toxin: protective antigen (PA, 83 kDa), lethal factor (LF, 90 kDa) and edema factor (EF, 90 kDa). These proteins play a key role in the pathogenesis of anthrax. EF and LF have enzymatic functions but require PA to achieve their biological effects.² Combined PA and LF, known as lethal toxin (LeTx), cause death when injected intravenously in animals. Edema factor (EF) associates with PA to produce edema toxin (EdTx) which when injected intradermally causes edema in the skin.³

Lethal factor is a zinc dependent metalloprotease which cleaves a specific bond in signaling proteins of the mitogen-activated protein kinase kinase family (MAPKK), destroying their ability to signal.^{4,5} Of the seven different MAPKKs the amino terminus of six are cleaved by LF.^{6,7} The crystal structure of LF complexed with the N-terminal portion of MAPKK-2 has been described.⁸

MAPKKide® (o-Abz/Dnp) is a synthetic peptide containing a cleavage site for anthrax lethal factor. It is a quenched fluorescent substrate peptide based on fluorescence resonance energy transfer (FRET). Initially, the N-terminally attached fluorophore, o-aminobenzoyl (Abz), is quenched by the C-terminally attached chromophore, 2,4 nitrophenyl (Dnp). Cleavage of the substrate by LF releases the fluorophore and full fluorescence is restored. The increase in fluorescence intensity is directly proportional to the amount of cleavage that has occurred and thus allows for accurate measurement of LF. The hydrolysis of the Abz-peptidyl-Dnp substrates can be followed using an excitation wavelength of 320 nm and an emission wavelength of 420 nm. MAPKKide® with the FRET pair, DABCYL/FITC is also available. Hydrolysis is followed using 490 nm and 523 nm as the excitation and emission wavelengths, respectively.

These substrates can be used for development of highly sensitive and rapid *in vitro* methods for screening and characterizing of LF inhibitors, which are potential therapeutic agents.⁹⁻¹³ List Biological Laboratories, Inc. provides custom screening services upon request.

MAPKKide® is supplied as a lyophilized powder, and a lot analysis detailing purity and reaction conditions accompanies each shipment. A calibration peptide, which is the cleavage product of MAPKKide® (o-Abz/Dnp) containing only the Abz at the N-terminal, is also available. A modified MAPKKide® called MAPKKide® Plus*, Product #532, is a fluorescently labeled peptide substrate specific for anthrax lethal factor (LF) and resistant to cleavage by nonspecific proteases found in plasma. This substrate is highly sensitive to LF and may be used to detect early anthrax infections in plasma.

* Patent pending

These products are intended for research purposes only and are not for use in humans or as diagnostic agents. For further information, please contact List Biological Laboratories.

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Related Products

| Product No. | Description | Size |
|-------------|---|-------------------------------|
| 530 | MAPKKide® Peptide Substrate (o-Abz/Dnp) for <i>Bacillus anthracis</i> LF | 200 nmoles |
| 531 | MAPKKide® Peptide Substrate (DABCYL/FITC) for <i>Bacillus anthracis</i> LF | 200 nmoles |
| 532 | MAPKKide® Plus Specific Substrate for Anthrax Lethal Factor | 100 nmoles |
| 539 | MAPKKide® Unquenched Calibration Peptide for Product #530 | 50 nmoles |
| 171 | Anthrax Protective Antigen (PA), Recombinant from <i>Bacillus anthracis</i> | 50 µg, 0.1 mg 0.5 mg, 1 mg |
| 172 | Anthrax Lethal Factor (LF), Recombinant from <i>Bacillus anthracis</i> | 70 µg, 0.1 mg, 1 mg, 10 mg |

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Posters (available at www.listlabs.com under each products' purchasing page)

- N. Shine, L. Eaton, K. Crawford (2003) *Internally Quenched Fluorogenic Substrates for Anthrax Lethal Factor*.
- N. Shine and K. Suryadi (2016) *Substrates for Specific and Quantitative Detection of Anthrax Lethal Factor in Plasma*.