DATA SHEET

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] Com- bined 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 mitogenactivated protein kinase kinase family (MAPKK), destroying their ability to signal.[4,5] LF cleaves the amino terminus of MAPKKs. Of the seven different MAPKKs, six are cleaved by LF. [6,7] The crystal structure of LF complexed with the N-terminal portion of MAPKK-2 has been recently described.[8] Within the cell, MAP kinase pathways are involved in the transduction of a variety of signals including those involved in cell proliferation and differentiation.[9]

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 1) detecting toxin contamination in food, clinical, and environmental samples; 2) monitoring the production of LF by fermentation processes; 3) detecting LF neutralizing antibodies, as well as 4) screening and characterizing of LF inhibitors, which are potential therapeutic agents. 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 Nterminal, is also available.

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

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Ordering Information

Product No.	Description	Size
530	MAPKKide® for fluorometric measurement ofLethal Factor (LF) activity	200 nmoles
531	MAPKKide® Peptide Substrate (o-Abz/Dnp) for *Bacillus anthracis* LF	200 nmoles
539	MAPKKide® Unquenched Calibration Peptide for Product #530	50 nmoles
171A	Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis*	0.1 mg
171B	Anthrax Protective Antigen (PA), Recombinant from *Bacillus anthracis*	1.0 mg
172A	Anthrax Lethal Factor (LF), Recombinant from *Bacillus anthracis*	0.1 mg
172B	Anthrax Lethal Factor (LF), Recombinant from *Bacillus anthracis*	1.0 mg

References

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