

MAPKKide® Plus*

Specific Substrate for Anthrax Lethal Factor

Bacillus anthracis is regarded as a major biological warfare threat. The principal virulence factors are a γ -linked poly-D-glutamic acid (PGA) capsule and a three component exotoxin composed of protective antigen (PA), lethal factor (LF) and edema factor (EF). These proteins act in binary combinations. The complex of PA, the cell binding component, with the LF enzyme, is termed lethal toxin and can cause death.^{1,2} PA and the enzymatic EF together cause skin edema.³ Secreted PA is cleaved by membrane peptidases.⁴ This allows the 63 kDa carboxy terminal fragment to oligomerize to a heptamer or higher.⁵ Cleavage of PA is an essential step in exposing the binding sites for EF and LF. The complex enters the cell through endocytosis.⁶ PA mediates the transfer of LF and EF to the cytoplasm where these enzymes recognize and alter their targets.

There are three forms of human anthrax: cutaneous, inhalation, and gastrointestinal. With the inhalational anthrax incident which resulted from the intentional release of anthrax spores in the bioterrorism attacks of 2001, early symptoms were similar to those for common illnesses.⁷ Once symptoms are severe and diagnosis is possible, the levels of toxins are dangerously high. The incubation period can vary from hours to weeks depending on the dose received. Once intoxication occurs, anthrax bacteria can multiply rapidly in the blood and begin to secrete significant quantities of the toxin.⁸ The inhalation form of *Bacillus anthracis* infection can kill quickly. While antibiotic treatment can clear the bacterium from the host, if diagnosis is delayed, the toxin, which is rapidly produced, may already be present in lethal amounts.

There is a critical need for a rapid, accurate, sensitive and simple assay to determine whether infection has occurred thereby allowing immediate treatment. Anthrax lethal factor (LF), an endopeptidase, is present in blood early in the infection.⁹ 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.^{10,11} Of the seven different MAPKKs the amino terminus of six are cleaved by LF.^{12,13} The crystal structure of LF complexed with the N-terminal portion of MAPKK-2 has been described.¹⁴

The use of peptidic substrates in plasma is problematic due to the presence of other proteases and the likelihood of nonspecific cleavage of the substrate. MAPKKide® Plus*, Product #532 is a fluorogenic peptide substrate containing 7-amido-4-methylcoumarin (AMC) which is specific for LF, while resistant to nonspecific proteases. Significantly, this newly designed substrate is highly sensitive to LF and may be used to detect early infections. Two methods for the detection of LF in plasma have been developed using this fluorogenic substrate: an HPLC-based and a microplate assay.

Briefly, the LF is enriched by capture from plasma using an LF antibody-coated microtiter plate, and the captured LF is then exposed to the MAPKKide® Plus* substrate. The amount of cleaved peptide substrate is determined by HPLC with fluorescence detection. Concentration of the LF using the antibody-coated plates allows for the detection of 5 pg LF/ml of neat plasma after 2 hours of incubation. Alternately the substrate may be added directly to diluted plasma and cleavage monitored by an increase in fluorescence as a function of time using a fluorescent microplate reader. The limit of detection by this simpler method is 1 ng LF/ml of plasma after 5 hours of digestion. Both methods can be confirmed by analysis of the reaction as a function of time.

MAPKKide® Plus* (AMC) is supplied as a lyophilized powder, and a lot analysis detailing purity and a protocol describing the procedure for the specific detection of active infection of *Bacillus anthracis* in plasma accompanies each shipment.

* 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* (AMC) Specific Substrate for Anthrax Lethal Factor	100 nmoles
539	MAPKKide® Unquenched Calibration Peptide for Product #530	50 nmoles
169 A,B	Anthrax Lethal Factor (LF-A), Recombinant from <i>Bacillus anthracis</i> Native Sequence	0.1 mg, 1 mg
171 D,E	Anthrax Protective Antigen (PA), Recombinant from <i>Bacillus anthracis</i>	50 µg, 0.5 mg
172 B,C,D	Anthrax Lethal Factor (LF), Recombinant from <i>Bacillus anthracis</i>	70 µg, 1 mg, 10mg

* Patent Pending

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