



CERTIFICATE OF ANALYSIS
MAPKKide® (o-Abz/Dnp)
Peptide Substrate for Anthrax Lethal Factor
Lot #5303A5

Contents:

Each vial of the Lethal Factor (LF) substrate, MAPKKide® (o-Abz/Dnp), contains 200 nmoles of lyophilized peptide. MAPKKide® (o-Abz/Dnp) is derived from the MAPKK-2 substrate for LF. N-terminal analysis of the digest product indicates that cleavage of MAPKKide® by LF occurs at the expected single specific site (Pro-Ala) in the MAPKKide® substrate. The solubility is at least 2 mM in water. Higher concentrations may be achieved in DMSO. In order to maximize recovery from the vial and stability on storage, stock solutions may be prepared in DMSO. This peptide is intramolecularly quenched by fluorescence resonance energy transfer (FRET). The N-terminally-linked fluorophore is o-aminobenzoic acid (o-Abz), and the acceptor chromophore is 2,4-dinitrophenol (Dnp). A control peptide, which is the cleavage product of MAPKKide® containing only the o-Abz at the N-terminal, is also available. **NOTE:** MAPKKide®, containing a DABCYL/FITC FRET pair, is available.

Reconstitution:

A small amount of peptide has been lyophilized in each vial. During lyophilization and transportation, this material may be distributed throughout the vial. Since it is common practice to reconstitute peptide in a small volume of solvent, we suggest visually locating the peptide and, if necessary, shaking it to the bottom of the vial prior to adding the solvent.

Concentration:

Peptide content is determined from amino acid analysis.

Purity:

The peptide is >90% pure as determined by reverse phase HPLC. The expected molecular weight is obtained by mass spectrometry.

Assay Conditions and Parameters for Utilizing MAPKKide® FRET Peptide:

Anthrax Lethal Factor, Recombinant (LF), Product #172A

LF is reconstituted in the reaction buffer for hydrolysis of MAPKKide® by LF: 20 mM HEPES, pH 8.2. Our data suggest that addition of 1 mg/ml BSA is beneficial to the stability and storage of LF at -20°C. Addition of ZnCl₂ or CaCl₂ has an inhibitory effect. The pH 8.2 HEPES buffer was obtained by titrating the free acid form of HEPES with the potassium salt form of HEPES. Concentrations of LF between 2 nM and 10 nM can be used depending on the instrumentation and experiment.

(continued)

MAPKKide® (o-Abz/Dnp), Product #530

Prepare a 5 mM stock solution of this peptide in DMSO as follows: Add 40 μ l of DMSO, Pierce cat. #20684, to a vial containing 200 nmoles of peptide. The resulting stock solution is 5 nM. Cover the vial with foil to protect from light and store frozen at -20°C.

For assays with LF, the 5 mM stock solution is diluted in 20 mM HEPES, pH 8.2, prior to use. When using a 96-well plate and a final volume of 250 μ l/well, a 250 μ M stock solution is convenient to use. The final concentration of MAPKKide® to be used is typically between 5 μ M and 10 μ M/well, depending on the instrumentation and experiment. Since DMSO inhibits cleavage, final concentrations to be used should be less than 1.25% of the total volume.

These FRET assays are run at 37°C. Excitation wavelength is 320 nm and emission wavelength is 420 nm. There is a linear dependence of fluorescence intensity on concentration of totally cleaved substrate up to ~30 μ M MAPKKide® (o-Abz/Dnp).

When measuring kinetic parameters such as the K_m and V_{max} for this FRET substrate, the data must be corrected for a phenomenon known as the "inner filter effect". This effect, as well as a method to determine an appropriate correction factor, is explained in the paper by Liu, *et.al.* (1999) in *Analytical Biochemistry*, **267**, 331-335. Since the fluorescence efficiency for the free o-Abz may be higher than for o-Abz when it is bound to the peptide, the use of the product #539, MAPKKide® Unquenched Calibration Peptide for #530, in the place of the free o-Abz, is suggested. This peptide contains the o-Abz bound to the N-terminal cleaved fragment of MAPKKide®.

Packing/Storage:

This lyophilized powder is stoppered under vacuum. It is recommended that it be stored at -20°C, protected from light. After reconstitution, aliquot and store at -20°C.

Handling:

This product is not known to be hazardous. Good laboratory technique should be employed in handling of this product. This requires observing the following practices:

1. Wear appropriate laboratory attire including a lab coat, gloves and safety glasses.
2. Do not mouth pipette, inhale, ingest or allow to come into contact with open wounds. Wash thoroughly any area of the body which comes into contact with the product.
3. Avoid accidental autoinoculation by exercising care when handling in conjunction with any injection device.
4. This product is intended for research purposes only. It is not intended for use in humans. List Biological Laboratories, Inc. is not liable for any damages resulting from the misuse or handling of this product.

FOR RESEARCH PURPOSES ONLY. NOT FOR USE IN HUMANS.

Approved: EE Date: 12/15/08

Approved: NS Date: 12-15-08