

DESCRIPTION

Source	<i>E. coli</i> -derived viral wnvNS3 Protease protein		
	MHHHHHHH	NS2b (Ser1423-Lys1470) (Ser1435Thr) Accession # YP_001527877.1	GGGGSGGGG
			West Nile Virus NS3 (Gly1506-Leu1689) Accession # YP_001527877.1
			GGGGSGGGG
			West Nile Virus NS3 (Gly1506-Leu1689) Accession # YP_001527877.1
	N-terminus		C-terminus
N-terminal Sequence Analysis	Met and Gly		
Predicted Molecular Mass	27 kDa & 20 kDa		

SPECIFICATIONS

SDS-PAGE	32 kDa and 18-22 kDa, under reducing conditions.
Activity	Measured by its ability to cleave the fluorogenic peptide substrate pERTKR-AMC (Catalog # ES013). The specific activity is >750 pmol/min/μg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Lyophilized from a 0.2 μm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials	<ul style="list-style-type: none"> Assay Buffer: 50 mM Tris, 30% (v/v) Glycerol, pH 9.5 Recombinant Viral wnvNS3 Protease (Catalog # 2907-SE) Substrate: L-PYROGlu-Arg-Thr-Lys-Arg-AMC (pERTKR-AMC) (Catalog # ES013) F16 Black Maxisorp Plate (Nunc, Catalog # 475515) Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent
Assay	<ol style="list-style-type: none"> Dilute rwnvNS3 Protease to 1 ng/μL in Assay Buffer. Dilute Substrate to 40 μM in Assay Buffer. In a plate load 50 μL of 1 ng/μL rwnvNS3 Protease, and start the reaction by adding 50 μL of 40 μM Substrate to wells. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of 40 μM Substrate. Read at excitation and emission wavelengths of 380 nm and 460 nm (top read), respectively, in kinetic mode for 5 minutes. Calculate specific activity: $\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* \text{ (RFU/min)} \times \text{Conversion Factor}^{**} \text{ (pmol/RFU)}}{\text{amount of enzyme (}\mu\text{g)}}$ <p>*Adjusted for Substrate Blank **Derived using calibration standard 7-Amino, 4-Methyl Coumarin (AMC) (Sigma, Catalog # A-9891).</p>
Final Assay Conditions	<p>Per Well:</p> <ul style="list-style-type: none"> rwnvNS3 Protease: 0.05 μg Substrate: 20 μM

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 200 μg/mL in sterile, deionized water.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	<p>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</p> <ul style="list-style-type: none"> 6 months from date of receipt, -20 to -70 °C as supplied. 3 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Infection of mosquito-borne West Nile Virus can cause severe neurological disease and can be epidemic. Two non-structural proteins, NS3 and NS2b, play an essential role in viral replication and are therefore a potential target for treatment and prevention of West Nile Virus disease. NS3 consists of a trypsin-like serine protease with a catalytic triad (His51, Asp75, Ser135) and a putative helicase. Requiring NS2b as the co-factor, NS3 protease processes viral polyprotein precursor (1, 2). The purified recombinant protein consists of three forms: the full-length fusion protein, the N-terminal NS2b, and the C-terminal NS3 with the G₄SG₄ linker. NS3 protease has a relatively narrow substrate specificity that prefers Arg in P1 and Lys in P2. The purified recombinant protein has autocatalytic activity that can lead to protein degradation. It is therefore important to store the sample below -20 °C and to keep on ice while working with the sample.

References:

1. Nall, T.A. *et al.* (2004) J. Biol. Chem. **279**:48535.
2. Chappell, K.J. *et al.* (2005) J. Biol. Chem. **274**:2896.