Product Data Sheet

Product Name:	HCV Protease FRET Substrate (RET S1)
Catalog Number:	AS-22991 (1 mg) Lot Number: See label on vial AS-22991-025 (0.25 mg) AS-22991-50 (50 mg) AS-22991-100 (100 mg)
Sequence:	Ac-Asp-Glu-Asp(EDANS)-Glu-Glu-Abu-ψ-[COO]-Ala-Ser-Lys(DABCYL)- NH2 (3-letter code) Ac-DE-D(Edans)-EE-Abu-ψ-[COO]-AS-K(Dabcyl)-NH2 (1-letter code)
Molecular Weight:	1548.6
% Peak Area by HPLC:	≥95
Appearance:	Lyophilized red powder

Peptide Reconstitution: Use distilled or higher quality water. Add water directly to the lyophilized peptide powder to obtain a final concentration of approximately 0.5 mg/mL to 1mg/mL or less. Gently vortex to mix. For peptides that have poor solubility in the suggested solvent, brief sonication may increase solubility in some cases.

Storage: Peptide is shipped at ambient temperature. Upon receipt, store lyophilized powder at –20°C or lower. Reconstituted peptide should be aliquoted into several freezer vials and stored at –20°C or lower. Do not freeze thaw.

Description: This peptide is a HCV protease substrate incorporating an ester bond between residues P1 and P1. Due to ready transesterification of the scissile bond to the acyl-enzyme intermediate, this substrate shows very high kcat/Km values, enabling detection of activity with subnanomolar nonstructural protein 3 (NS3 protease) concentrations. It is widely used for the continuous assay of NS3 protease activity. Substrate cleavage is proportional to the enzyme concentration with a detection limit for NS3 between 1 nM and 250 pM. Upon cleavage of this substrate, fluorescence can be monitored at Abs/Em = 355/500 nm. Ref: Lin, C. et al. *J Biol Chem* **10**, 1074 (2004); Kakiuchi, N. et al. *J Virol Methods* **80**, 77 (1999); Liu, Y. et al. *Anal Biochem* **267**, 331 (1999); Taliani, M. et al. *Anal Biochem* **240**, 60 (1996).

Additional Information: *Listed below are relevant information that may provide a guideline on how to use this product. End users will have to adapt to their own specific applications.*

The sensitivities of the NS3 protease domain variants to telaprevir were determined in microtiter plates by using an internally quenched fluorogenic depsipeptide, RET-S1 {acetyl-DED(EDANS)EEAbuψCOO]ASK (DABCYL)-NH2; Anaspec Incorporated, San Jose, CA}. Briefly, the NS3 protease domain was preincubated with 5 μM KK4A peptide for 10 min at 25°C and for another 10 min at 30°C. Then, the protease mixture was incubated with telaprevir for 60 min at 30°C and for another 20 min at 30°C with 5 μM RET-S1 substrate, and product release was monitored- Zhou, Y. et al. *Antimicrob Agents Chemother* **52**, 110 (2008).

Sensitivity of the NS3 protease domain variants to telaprevir was determined in 96-well microtiter plates (NBS 3990; Corning Glass) using an internally quenched fluorogenic depsipeptide, RET-S1 (DABCYL) (Anaspec Inc., San Jose, CA). Briefly, the NS3 protease domain was preincubated with 5 μM KK4A peptide in 50 mM HEPES (pH 7.8), 100 mM NaCl, 20% glycerol, 5 mM dithiothreitol at 25 °C for 10 min and at 30 °C for 10 min. Telaprevir, serially diluted in Me2SO, was added to the protease mixture and incubated for an additional 60 min at 30°C. The reaction was started by the addition of 5 μM RET-S1 substrate and incubated at 30 °C. Product release was monitored for 20 min (excitation at 360 nm and emission at 500 nm) in a

Tecan SpectraFluorPlus plate reader (Tecan US, Durham, NC)- Zhou, Y. et al. *J Biol Chem* **282**, 22619 (2007).

The peptide substrate NS5A/5B (H-EDVVAbuCSMSY-OH) and the fluorescent substrate RET-S1 (Ac-DED[EDANS]EEAbuψ [COO]ASK[DABCYL]-NH2) were bought from AnaSpec, Inc., (San Jose, CA). The assay was performed in a buffer containing 50 mM HEPES (pH 7.8), 100 mM NaCl, 20% glycerol, and 5 mM dithiothreitol (buffer A), using the RET-S1 fluorescent peptide as the substrate. Reactions were continuously monitored using an fMax fluorescence microtiter plate reader (Molecular Devices, Sunnyvale, CA) thermostatted at 30°C, with excitation and emission filters of 355 nm and 495 nm, respectively. A stock solution of HCV NS3 protease in buffer A containing 25 μM KK4A peptide was preincubated for 10 min at room temperature, followed by an additional 10 min of incubation at 30°- Perni, RB. et al. *Antimicrob Agents Chemother* **50**, 899 (2006).

An internally quenched fluorogenic depsipeptide (FRET substrate), Ac-DED(EDANS)EE Abu ψ[COO]ASK (DABCYL)-NH2, was purchased from AnaSpec Inc. (San Jose, CA). The assay was run in a continuous mode in a 96-well microtiter plate format. The buffer was composed of 50 mM HEPES (pH 7.8), 100 mM NaCl, 20% glycerol, 5 mM dithiothreitol, and 25 μM KK4A peptide (KKGSVVIVGRIVLSGK). The KK4A peptide represents the central region of the NS4A cofactor from genotype 1a with lysine residues added for improved solubility. The reaction was initiated by the addition of the FRET substrate after a 10-min preincubation of the buffer components with a 2 nM concentration of the NS3 protease at room temperature- Lin, C. et al. *J Biol Chem* **279**, 17508 (2004).

Published Citations:

Beran, R. et al. *J Virol* **83**, 3268 (2009).
Lin, T. et al. *Antimicrob Agents Chemother* **53**, 1377 (2009). Pan, K. et al. *Antimicrob Agents Chemother* **10** (2009). Phan, T. et al. *J Virol* **83**, 8379 (2009).
Beran, R. et al. *J Biol Chem* **283**, 29929 (2008).
Zhou, Y. et al. *Antimicrob Agents Chemother* **52**, 110 (2008). Zhou, Y. et al. *J Biol Chem* **282**, 22619 (2007).
Zhou, Y. et al. *Antimicrob Agents Chemother* 10.1128/AAC.00863-07 (2007). Perni, RB. et al. *Antimicrob Agents Chemother* **50**, 899 (2006).
Ferreon, J. et al. *J Biol Chem* **280**, 20483 (2005). Lin, C. et al. *J Biol Chem* **279**, 17508 (2004).

For Research Use Only