

FRETS-VWF73

*Fluorescence-Quenching Substrate
for ADAMTS-13*

Frets

Thrombotic thrombocytopenic purpura (TTP) is a rare and potentially fatal blood condition characterized by the formation of microvascular thrombi. Until recently the cause of TTP remained elusive. Latest research points to the involvement of a protein in the plasma called von Willebrand factor (vWF). vWF is an extremely large molecule that is able to bind to platelets or to injured blood vessel lining and is a normal component of plasma that is required for blood clotting. vWF molecules are rather sticky and are usually found circulating in the blood. Normally, they are broken down to slightly smaller sizes so that vWF retains its adhesive properties without binding inappropriately, leading to undesired blood clots. von Willebrand

disease is an inherited condition characterized by excessive bleeding and is due to lack of vWF. In TTP, vWF is synthesised normally but its cleavage is defective. This is considered to be due to the lack of enzyme activity called vWF cleaving protease. The vWF cleaving protease has been reported to be a new member of the ADAMTS family of metalloproteases, designated as ADAMTS-13.

Currently, there is no specific test to confirm the diagnosis of this potentially fatal condition. A new fluorescent-quenching substrate is now available from the Peptide Institute and Peptides International. This ADAMTS-13 specific substrate should be a valuable tool to aid in the discovery of diagnostic markers for TTP.

CODE	FRETS-VWF73	QTY
SFR-3224-s	<p>FRETS-VWF73 (Trifluoroacetate Form) Asp-Arg-Glu-A₂pr(Nma)-Ala-Pro-Asn- Leu-Val-Tyr-Met-Val-Thr-Gly-A₂pr(Dnp)-Pro-Ala-Ser-Asp-Glu-Ile-Lys-Arg-Leu-Pro-Gly-Asp-Ile-Gln-Val-Val-Pro-Ile-Gly-Val-Gly-Pro-Asn-Ala-Asn-Val-Gln-Glu-Leu-Glu-Arg-Ile-Gly-Trp-Pro-Asn-Ala-Pro-Ile-Leu-Ile-Gln-Asp-Phe-Glu-Thr-Leu-Pro-Arg-Glu-Ala-Pro-Asp-Leu-Val-Leu-Gln-Arg A₂pr(Nma): N^β- [2-(Methylamino)benzoyl]- 2,3- diaminopropionic acid A₂pr(Dnp): N^β-(2,4-Dinitrophenyl)-2,3-diaminopropionic acid (M.W. 8314.30) C₃₇₀H₅₈₃N₁₀₃O₁₁₃S <i>Fluorescence-Quenching Substrate for ADAMTS-13</i></p> <ul style="list-style-type: none"> This compound is produced by Peptide Institute, Inc. under license of the National Cardiovascular Center in Japan. Japanese patent No. 3944586, US patent application No: US-2007-0065895-A1. 	0.1 mg vial

- 1) K. Kokame, M. Matsumoto, Y. Fujimura, and T. Miyata, *Blood*, **103**, 607 (2004). (*VWF73 Sequence*)
- 2) K. Kokame, Y. Nobe, Y. Kokubo, A. Okayama, and T. Miyata, *Br. J. Haematol.*, **129**, 93 (2005). (*FRETS-VWF73*)

Lot Specific Data - see vial insert for detailed instructions

FRETS-VWF73 SFR-3224-s Lot No. 550116
(Trifluoroacetate Form)

Asp-Arg-Glu-A₂pr(Nma)-Ala-Pro-Asn-Leu-Val-Tyr-Met-Val-Thr-Gly-A₂pr(Dnp)-Pro-Ala-Ser-Asp-Glu-Ile-Lys-Arg-Leu-Pro-Gly-Asp-Ile-Gln-Val-Val-Pro-Ile-Gly-Val-Gly-Pro-Asn-Ala-Asn-Val-Gln-Glu-Leu-Glu-Arg-Ile-Gly-Trp-Pro-Asn-Ala-Pro-Ile-Leu-Ile-Gln-Asp-Phe-Glu-Thr-Leu-Pro-Arg-Glu-Ala-Pro-Asp-Leu-Val-Leu-Gln-Arg A₂pr(Nma): N^β-[2-(Methylamino)benzoyl]-2,3-diaminopropionic acid
A₂pr(Dnp): N^β-(2,4-Dinitrophenyl)-2,3-diaminopropionic acid
(M.W. 8314.3) C₃₇₀H₅₈₃N₁₀₃O₁₁₃S

Fluorescence-Quenching Substrate for ADAMTS-13

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This vial contains exactly **0.11 mg (14 nmol)** of the titled compound, which has been lyophilized as an amorphous powder from aqueous trifluoroacetic acid.

Storage (solution) The aqueous DMSO solution is stable at -20 °C for more than one month if it is kept in the dark.
(powder) The undissolved peptide should be stored at -20 °C in the dark.

Solution Carefully open the cap. Pour 35 μl of DMSO* (analytical grade) into the vial followed by 105 μl of distilled water. Close the cap tightly. Shake well until all the contents are dissolved. If necessary, sonicate the prepared solution gently. This procedure furnishes a 100 μM solution of the titled compound.

*DMSO: dimethyl sulfoxide

Caution: This peptide is sold for research purposes only and not for use in humans.

Reaction Buffer 5 mM Bis-Tris, 25 mM CaCl₂, and 0.005% Tween-20 at pH 6.0

Reagents

- 1) Substrate solution: Dilute the stock solution of Code SFR-3224-s. 25-fold with the reaction buffer
- 2) Standard plasma solutions (for standard curve): Dissolve 0, 1, 2, 3, 4, 5, and 6 μl of normal human plasma* in 100 μl of the reaction buffer
- 3) Test plasma solution: Dissolve 4 μl of test plasma* in 100 μl of the reaction buffer

* Both plasma should be pre-anti-coagulated with sodium citrate.

Procedure

- 1) Prepare 100 μl of the standard and test plasma solutions in each well of a 96-well white plate at room temperature
- 2) Add 100 μl of the substrate solution into the prepared solution in each well (final substrate concentration at 2 μM)
- 3) Set the plate in a fluorescence spectrophotometer [such as Wallac 1420 ARVO (PerkinElmer)] with λ_{ex} = 340 nm and λ_{em} = 450 nm at 30 °C
- 4) Measure fluorescence of each well every five minutes between 0 and 60 min.
- 5) Calculate the reaction rate (slope) by linear regression of fluorescence

