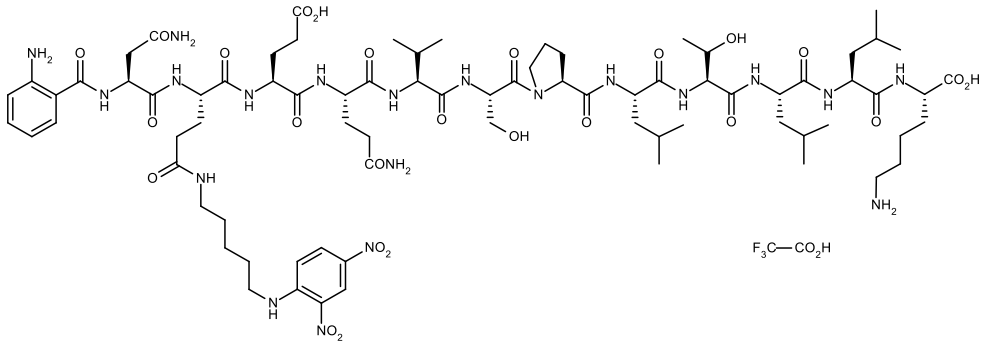
Product Data Sheet

Product number **A101**
Revision number RN6.0

Product Name	FXIII-Assay Substance, fluorescent Abz-NE(CAD-DNP)EQVSPLTLLK-OH trifluoroacetate
Molecular Formula	$C_{80}H_{123}F_3N_{20}O_{27}$ (free base: $C_{78}H_{122}N_{20}O_{25}$)
Molecular Weight	1853.95 (free base: 1739.92)
Chemical Structure	
Purity by HPLC	>95 % (214 nm)
Solubility	50 µM in 0.1% (v/v) DMSO / aqueous buffers Pre-dissolve e.g. 10 mg (5.39 µmol) in 108 µl DMSO - dilute e.g. 10 µl of that stock solution (50 mM) with 9990 µl aqueous buffer to obtain a 50 µM solution. DMSO stock solutions are sterile and can be stored at -20°C for at least 6 months. To avoid too many freeze-thaw cycles, we strongly recommend storage of aliquots.
Appearance	Yellow solid
Storage	Store at -20°C, desiccate
Related products	F004 Fibrinogen human plasma T007 Coagulation factor XIII human plasma T027 Human blood coagulation Factor XIII, recombinant
Reference(s)	Oertel, K. *et al. Anal. Biochem.* **2007**, *367*, 152. Dodt, J. *et al. Anal. Biochem.* **2013**, *439*, 145. Berny-Lang, M.A. *et al. J. Am. Heart Assoc.* **2013**, *2*, e000026. Schroeder, V. *et al. Br. J. Haematol.* **2015**, *168*, 757. de Jager, M. *et al. Neuropathol. Appl. Neurobiol.* **2016**, *42*, 255. Király, R. *et al. Amino Acids* **2016**, *48*, 31. van der Wildt, B. *et al. Nucl. Med. Biol.* **2016**, *43*, 232. Dodt, J. *et al. Br. J. Haematol.* **2016**, *172*, 452. Chrobok, N.L. *et al. PLoS ONE* **2018**, *13*, e0209522. Akbar, A. *et al. J. Med. Chem.* **2017**, *60*, 7910-7927. Singh, S. *et al. Int. J. Mol. Sci.* **2019**, *20*, 2682.
Release date	29 November 2021
NOTE	With respect to PDS RN4.0 the molecular weight was corrected to 1853.95, taking into account that A101 traps only one TFA counter ion instead of two counter ions as stated in former PDS versions. The presence of one TFA counter ion is valid for all former batches, too.

Background: During the course of our continuous quality assurance process, Zedira developed a novel method to quantify the TFA counter ion content by using a ^{19}F NMR method according to Little *et al.* (*J. Pharm. Biomed. Anal.* **2007**, *43*, 1324), based on an internal standard.

Please note that neither the synthesis and purification nor the product as such has been changed. Please consider molecular weight correction if necessary.

INTENDED FOR RESEARCH USE ONLY, NOT FOR USE IN HUMAN, THERAPEUTIC OR DIAGNOSTIC APPLICATIONS.

Factor XIII assay:

- **Buffer (1 Litre):**

6.88 g	Tris	56.8 mM
1.26 g	$CaCl_2$	11.36 mM
6.64 g	NaCl	113.6 mM
1.13 g	PEG 8000	0.113 % (w/v)
0.713 g	H-Gly-OMe*HCl	5.68 mM
5.68 mg	Hexadimethrinbromide	5.68 mg/L

Dissolve the substances in water and adjust pH to 7.5 using HCl.

- **Substrate stock solution:**

10 mg Abz-NE(CAD-DNP)EQVSPLTLLK-OH (A101) were dissolved in 108 µl DMSO (please vortex thoroughly).
DMSO stock solutions can be stored at -20°C for at least 6 months. To avoid too many freeze-thaw cycles, we strongly recommend storage of aliquots. Please protect from light.

- **Assay solution:**

Dilute the substrate stock solution 1:1000 (v/v) with buffer. In case of plasma and other fibrin(ogen) containing samples, add 1.1 mg GPRP-NH_2*2HCl per ml assay solution to avoid clotting.
This assay solution should be adjusted to 37°C. Please protect from light.

- **Thrombin solution:**

Dissolve thrombin (lyophilised, 100 NIH U) in 100 µL buffer.

Assay condition:

Add 20 µl thrombin solution to 880 µL assay solution in a suitable fluorescence cuvette at 37 °C. Add 100 µL sample and mix thoroughly. Start measurement of fluorescence intensity imediately. The activity could be determined by the increase of flourescence between 5 to 10 minutes after activation by thrombin in comparison to standard plasma or purified factor XIII.

Final concentrations in the assay:

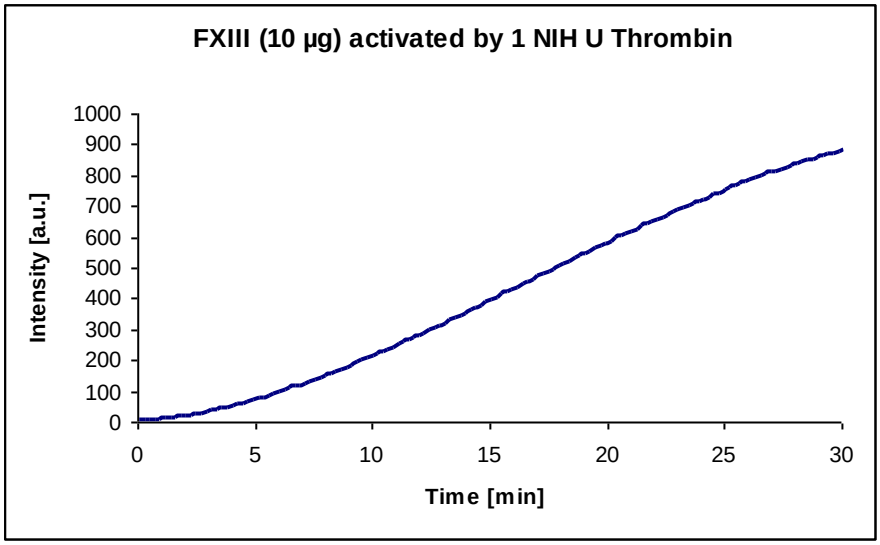
Tris-HCl (50 mM), $CaCl_2$ (10 mM), NaCl (100 mM), PEG_{8000} [0.1% (w/v)], H-Gly-OMe*HCl (5 mM), Hexadimethrinbromide (5 mg/L), A101 (50 µM), GPRP-NH_2*2HCl (2 mM) and thrombin (20 NIH-Units).

Instrument Parameters

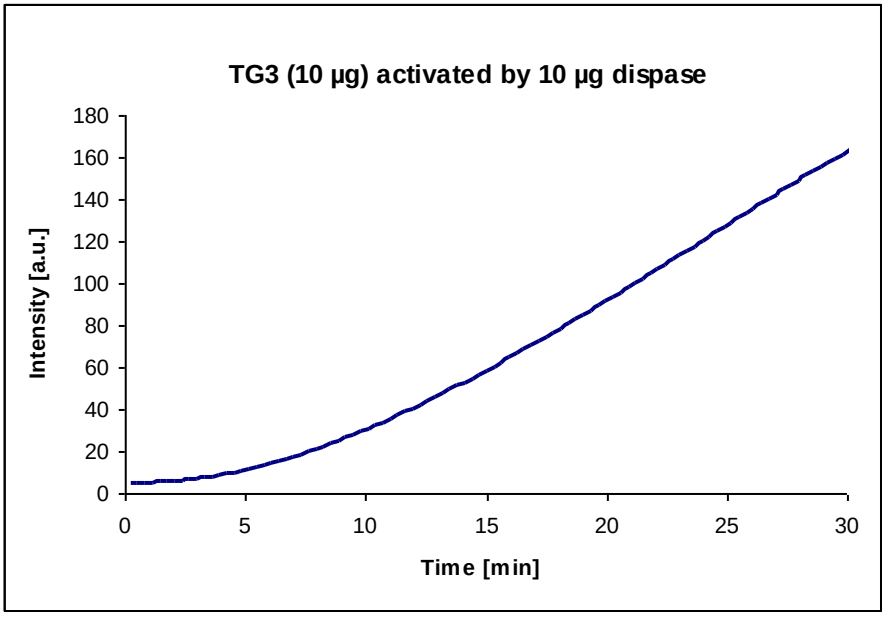
Ex. wavelength (nm)	313.00
Em. wavelength (nm)	418.00
Ex. Slit (nm)	5
Em. Slit (nm)	5
Averaging Time (s)	2.0000
Cycle time(min)	0.0000
Stop time(min)	15.0000
Emission filter	Open
Excitation Filter	Auto
PMT voltage (V)	Medium (600 V)

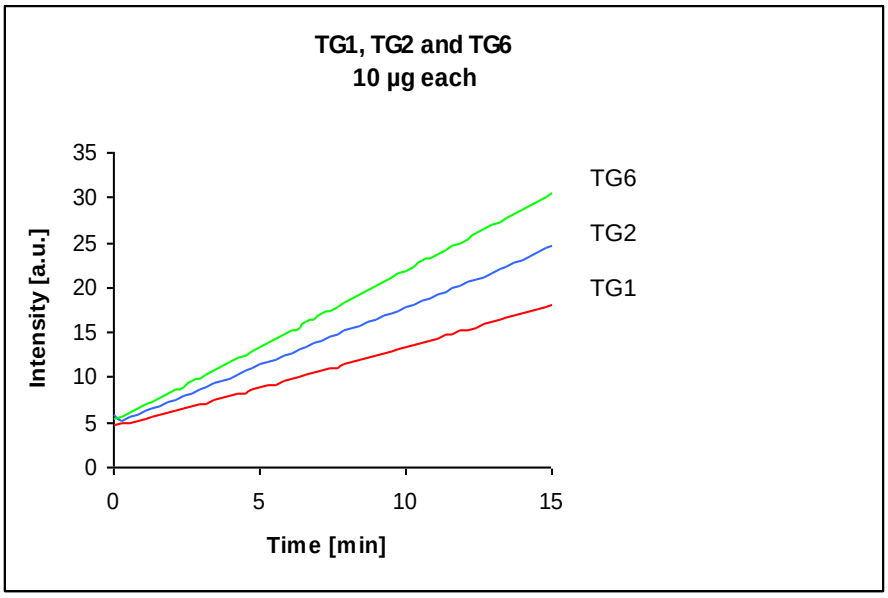
Remarks: Assay volume might be reduced to 200 µl when measuring in a suitable plate reader.

Example: On line monitoring of FXIII activation by Thrombin and increase of fluorescence intensity by FXIIIa iso-peptidase activity.



Example 2: Determination of other Transglutaminases using A101 in suitable buffers





Please notice that the Transglutaminases used display significant differences in fluorescense intensity. However, all purified Transglutaminases discribed can be assayed using A101. The concentration of A101 in the assay is 93 µg/ml.