Directions for use

ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT

Isopeptidase-activity based MTG assay
(Fluorogenic: λ_{ex} = 330 nm; λ_{em} = 418 nm)

Art.-No. F015

For Research & Development Only

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1. Assay principle

MICROBIAL TRANSGLUTAMINASE (MTG or synonymous BTG for Bacterial Transglutaminase) is a multifunctional enzyme best known for its crosslinking activity, but it can also cleave isopeptide bonds. This feature is used to provide an **easy to handle**, **robust, sensitive,** and **precise fluorogenic assay** to measure MTG activity. The assay is suitable for research purposes, drug discovery programs, and quality assurance.

MICROBIAL TRANSGLUTAMINASE (MTG) cleaves a dark quencher molecule from the side chain of a MTG specific peptide (A167, SUBSTRATE REAGENT). The MTG-substrate complex is cleaved by the incorporation of a glycine methyl ester molecule. Subsequently, the fluorescence of an N-terminal coupled fluorophore increases and can be continuously monitored (excitation wavelength 330 nm; emission wavelength 418 nm). The assay principle is shown in Figure 1.

2. Intended use

Specific and sensitive measurement of MICROBIAL TRANSGLUTAMIASE (MTG) activity. Suitable for determination of MTG concentrations of 2.5 nM to 80 nM (0.1 µg/mL to 3 µg/).

3. Test sample

Sample containing MICROBIAL TRANSGLUTAMINASE (MTG).

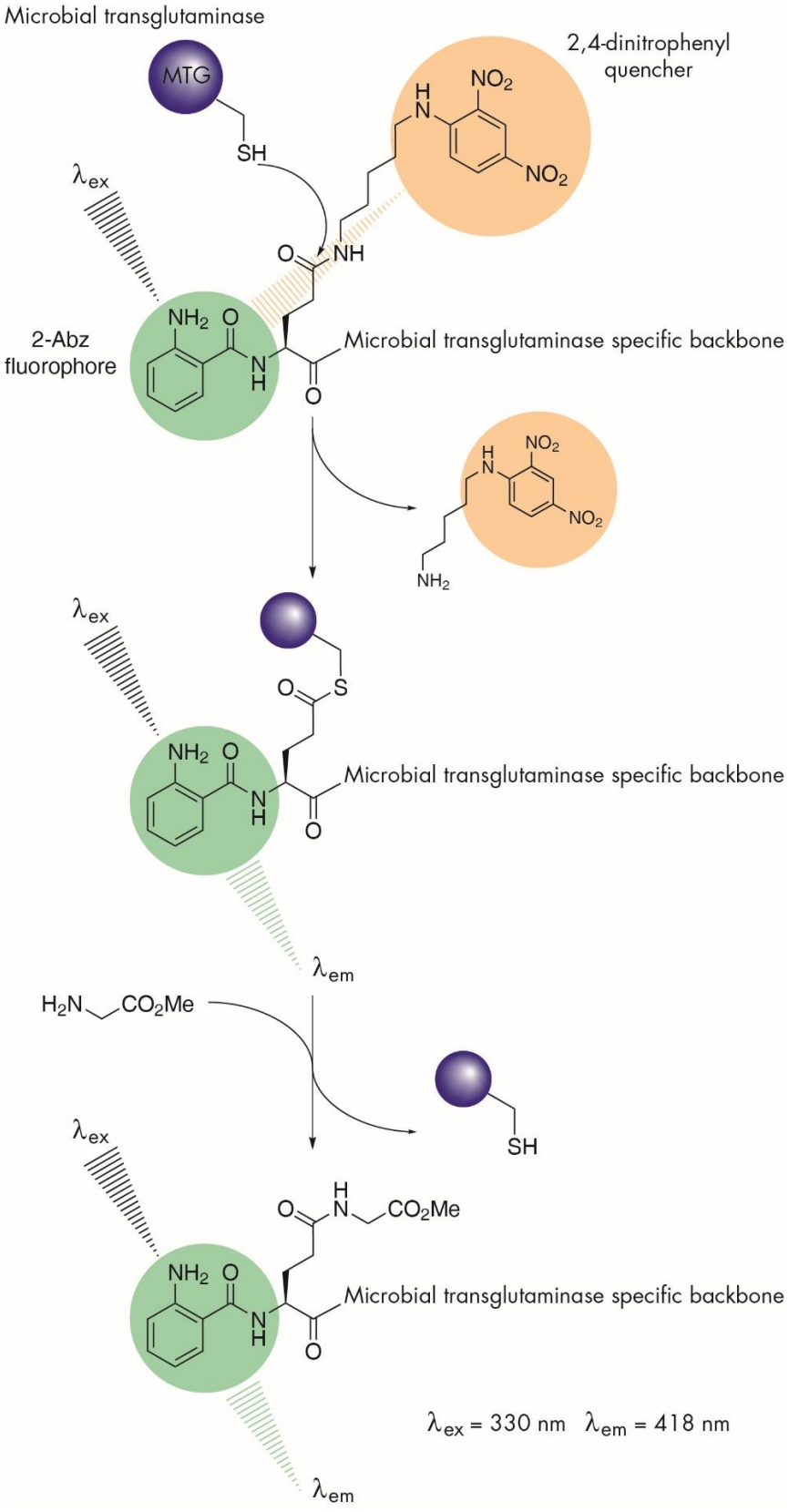


Figure 1: Cleavage of a carboxamide bond in the side chain of a specific peptidic backbone is catalyzed by MICROBIAL TRANSGLUTAMINASE (MTG). The release of the dark quencher is followed by incorporation of glycine methyl ester. After the dark quencher is released, the fluorescence of the fluorophore increases.

The assay continuously monitors MTG activity.

4. Reagents in the kit

(1) SUBSTRATE REAGENT (SR): 2 x 23 µL MTG specific assay peptide A167 (DMSO solution).

(2) BUFFER REAGENT (BR): 2 x 18 mL 200 mM TRIS buffer pH 6.0 containing 5.6 mM glycine methyl ester (lyophilizate).

(3) DILUTION BUFFER (DB): 1 x 18 mL 200 mM TRIS buffer pH 6.0 (lyophilizate).

Optionally available at Zedira (not included in the kit):

- T300: MTG REFERENCE, Andracon™, recombinant, HEPES-formulated, lyophilized
- T250: MTG Reference, Andracon™, recombinant, HEPES-formulated, (frozen) liquid
- A168 (ABZ-PEPTIDE-CALIBRATOR, reaction product formed for calibration purposes).
- C102 (MTG-inhibitor)

5. Reagent preparation, storage, and stability

ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT (BUFFER REAGENT and DILUTION BUFFER lyophilizates and SUBSTRATE REAGENT) must be stored at 2-8°C (shipment at ambient temperature is possible). The unopened reagents are stable according to the retest date printed on the box.

Table 1: Reconstitution of Kit components

Component	Preparation	Storage
BUFFER REAGENT (**BR**) 2 x 18 mL TRIS buffer lyophilizate (200 mM TRIS, 5.6 mM Gly-OMe, pH 6.0)	Add 18 mL of deionized water per vial and mix carefully	Consume within one day or store frozen at -20°C for at least 4 weeks
DILUTION BUFFER (**DB**) 1 x 18 mL TRIS buffer lyophilizate (200 mM TRIS, pH 6.0)	Add 18 mL of deionized water per vial and mix carefully	Consume within one day or store frozen at -20°C for at least 4 weeks
SUBSTRATE REAGENT (**SR**) 2 x 23 µL MTG specific assay peptide (DMSO solution)	Ready-to-use DMSO solution Bring to ambient temperature	Store at 2-8°C Stable for at least 6 months

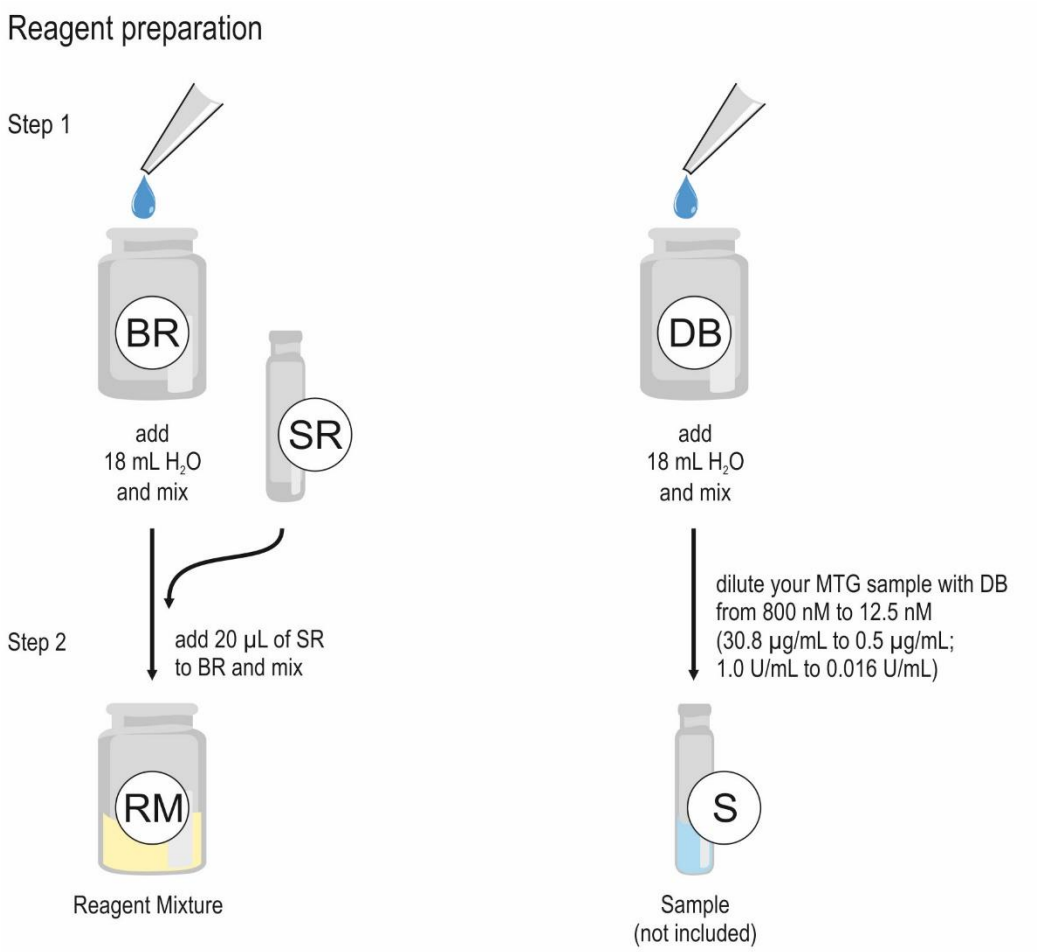
Table 2: Preparation of REAGENT MIXTURE (**RM**)

Component	Preparation	Storage
SUBSTRATE REAGENT (SR) BUFFER REAGENT (BR)	Add 20 µL **SR** to 18 mL prepared **BR** and mix	Consume within 2 hours Protect from light Keep **RM** at ambient temperature until usage

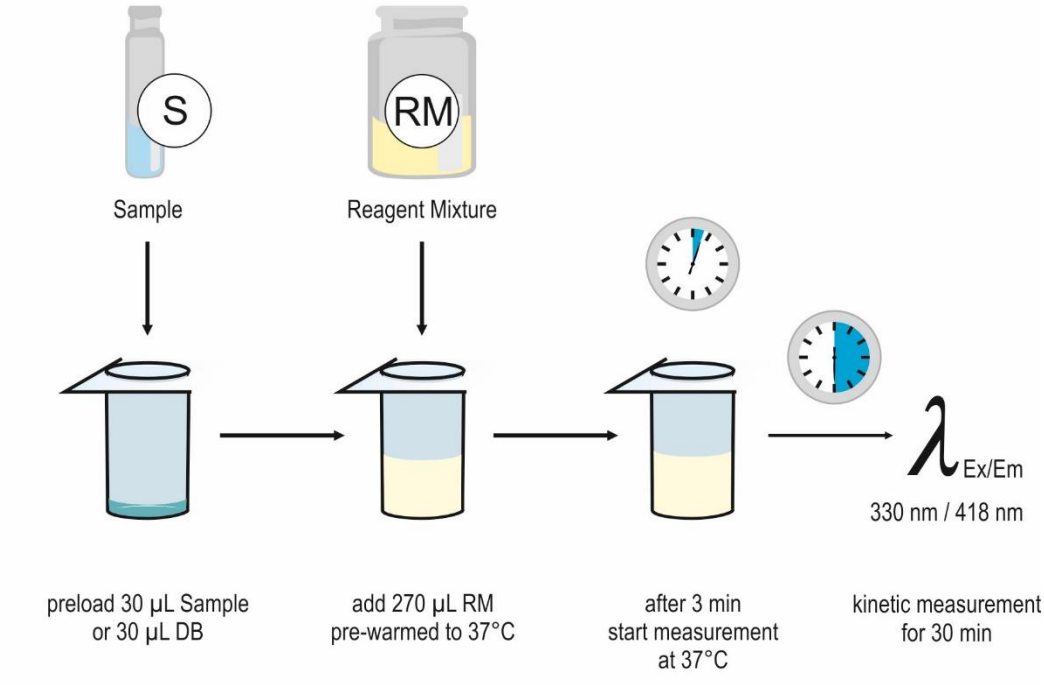
Table 3: Components optionally available at Zedira (not included in the kit)

Component	Preparation	Storage
T300 MTG REFERENCE, Andracon™ lyophilized	Add the volume of H_2O as indicated in the respective CoA. Rotate vial gently until solid dissolves Serial dilution 800-12.5 nM (30.8-0.5 µg/mL) in **DB** Final assay concentration will be one-tenth	Prepare enzyme diluent directly before use Do not vortex! Store diluent on ice
T250 MTG REFERENCE, Andracon™ liquid	Thaw product carefully at ambient temperature Serial dilution 800-12.5 nM (30.8-0.5 µg/mL) in **DB** Final assay concentration will be one-tenth	Prepare enzyme diluent directly before use Do not vortex! Store diluent on ice Concentrate is not susceptible to freeze-thawing, shown for ten freeze-thaw cycles
A168 ABZ-PEPTIDE-CALIBRATOR reaction product formed for calibration purposes	Prepare a DMSO stock solution or serial dilution according to your desired assay concentration as indicated in the respective PDS	DMSO stock solutions can be stored at -20°C for at least 6 months
C102 MTG-inhibitor	Prepare a DMSO stock solution or serial dilution according to your desired assay concentration as indicated in the respective PDS	DMSO stock solutions can be stored at -20°C for at least 6 months

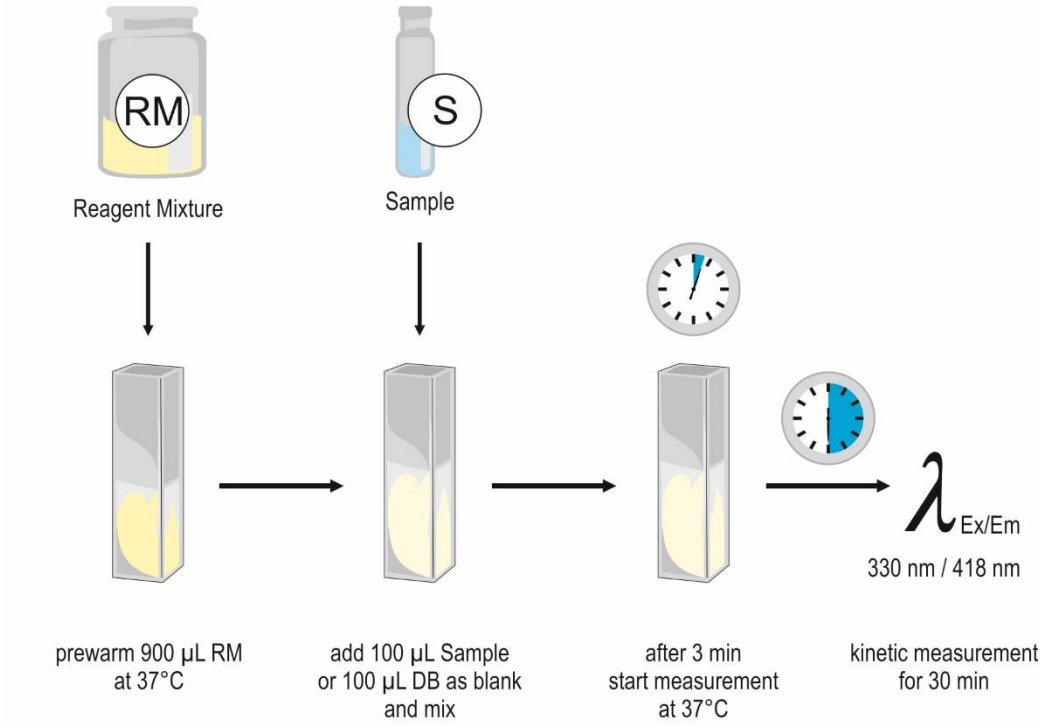
6. Schematic assay overview



Assay procedure (96 well microtiter plate)



Assay procedure (cuvette)



7. Procedure and Equipment

Set the fluorescence spectrophotometers temperature to 37°C, if applicable. Data shown in the manual are obtained at this temperature. However, the kit may also be run at ambient temperature.

The ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT (F015) can be used in fluorescence plate readers using microplates as well as in standard fluorescence spectrophotometers with cuvettes. Refer to the instructions of the manufacturer.

Add SAMPLE and REAGENT MIXTURE depending on your assay format:

Microtiter plate (96 well, 300 µL): Select a microplate that is rated for fluorescence-based assays and exhibits little or no autofluorescence in the emission range of the reagent you wish to use. Black plates are typically recommended.

Preload the wells with 30 µL of your SAMPLE. Start the reaction by adding 270 µL of prewarmed REAGENT MIXTURE to SAMPLE and mix thoroughly.

Fluorescence cuvette (1 mL): Start the reaction by adding 100 µL of SAMPLE to 900 µL of REAGENT MIXTURE, mix thoroughly.

Use DILUTION BUFFER instead of SAMPLE to generate a blank. Measurement of SAMPLES in duplicate or triplicate is recommended.

Start the kinetic measurement 3 minutes after starting the reaction using the instrument settings shown in table 4.

Table 4: Instrument settings for fluorescence spectrophotometer.

Excitation wavelength	330 nm
Emission wavelength	418 nm
Assay time (min)	30

Determine the slope of fluorescence increase over a reaction time of 30 min. Use the linear part of slope for assessment.

Unlike absorbance, fluorescence is not an absolute measurement. The RFU scale cannot be standardized. Accordingly, absolute counts cannot be compared between readers of different manufacturers. The intensity of a fluorescent signal is usually relative to other measurements, to a reference measurement, or to the gain settings [3].

Adjust fluorescence gain of the sample with the expected highest signal output (e.g. your positive control) to avoid saturation but still cover a good assay dynamic range.

NOTE: A MTG REFERENCE is optionally available (see section 4) for validating the kit in different laboratories and experimental settings to facilitate comparability of data. You may also consider using Zedira product A168 ("ABZ-PEPTIDE-CALIBRATOR"), the reaction product formed, for calibration purposes.

8. Number of sample measurements

The assay reagents per kit are sufficient for 130 measurements in microtiter plates and 38 measurements in cuvettes.

9. Results

Fluorescence increase is proportional (2nd polynomial fit) to the MICROBIAL TRANSGLUTAMINASE activity. The correlating MTG amount can be calculated with a reference curve. Figures 2 A and B show typical plots.

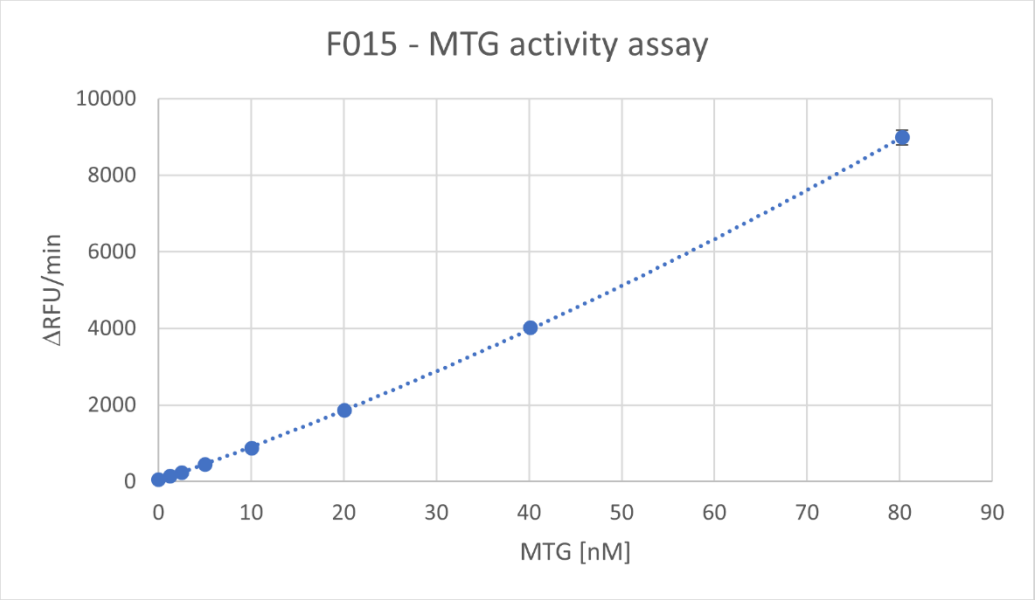


Figure 2A

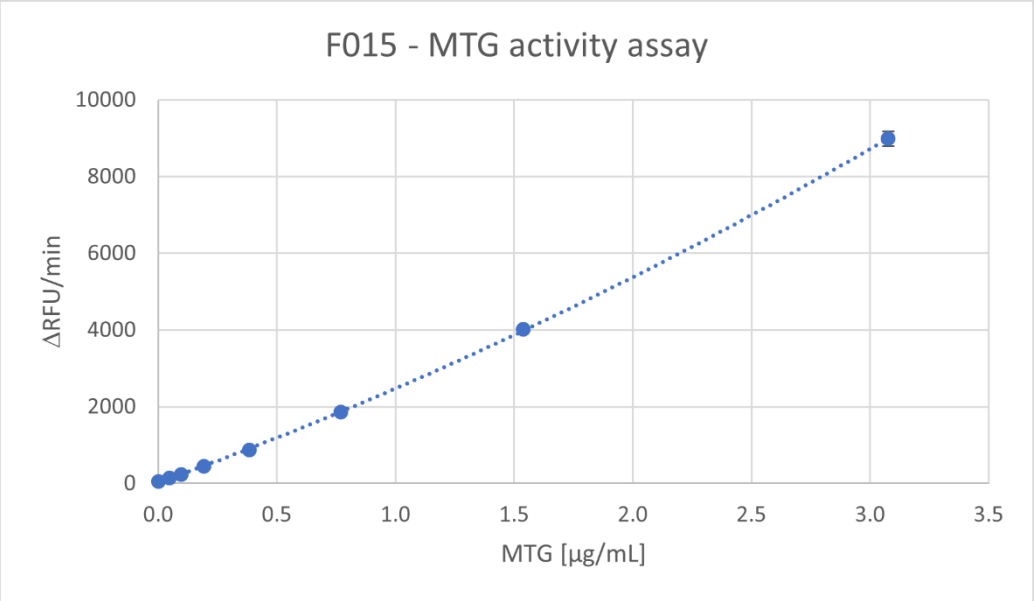


Figure 2B

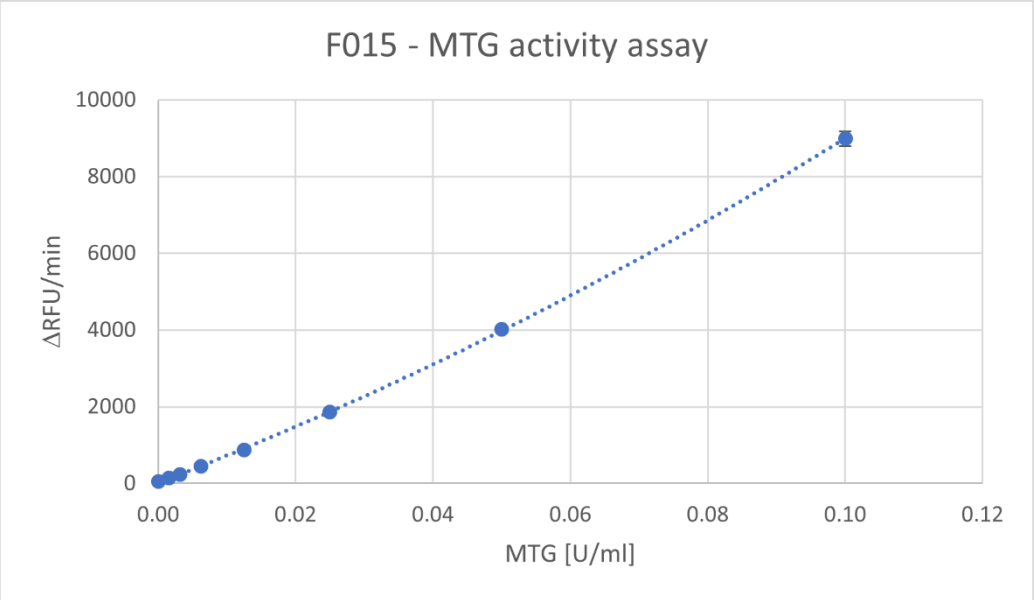


Figure 2C

Figure 2 A & B: Reference curves for recombinant MICROBIAL TRANSGLUTAMINASE (MTG), Reference Lot 2921aT001 (38.3 kDa, specific activity: 32.5 U/mg, 145.8 U/mL dissolved in H_2O according to the CoA (3).
Definition: One unit will catalyze the formation of 1 µmol of hydroxamate per min from Z-Gln-Gly-OH and hydroxylamine at pH 6.0 at 37°C, Grossowicz et al. (1950)

10.Reference Range

The ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT (F015) is suitable for measurements of 2.5 nM to 80 nM (0.1 µg/mL to 3 µg/mL) of MTG.

11.Kinetic parameters

The determination of MICROBIAL TRANSGLUTAMINASE activity is based on the ability of MTG to cleave isopeptide bonds. The kinetic parameters obtained for MTG specific peptidic substrate in ZEDI*XCITE* FLUOROGENIC MTG-ASSAY are summarized in table 5.

Table 5: Kinetic parameters of MTG substrate determined by ZEDI*XCITE* FLUOROGENIC MTG-ASSAY with MTG.

	K_M [µM]	k_{cat} [s^{-1}]	k_{cat}/K_M (M^{-1} s^{-1})
ZEDI*XCITE* substrate peptide	43.7	0.02	492.9

12.Limitations

The ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT (F015) is meant for research and development only. The kit has been optimized and valdated for the measurement of purified MTG in buffer.

13.Precision

The coefficient of variation determined during kit development was 3.5% for 80 nM (3.1 µg/mL) recombinant recombinant MICROBIAL TRANSGLUTAMINASE (MTG) using Reference Lot 2921aT001. Day to day variance was 0.44%. No interfering activities are known to the manufacturer. The Lower Limit of Quantification (LLOQ) is 2.5 nM (0.1 µg/mL).

References

[1] Parameswaran, K.N. *et al. J. Biol. Chem.* **1997**, *272*, 10311.

[2] Oertel, K. *et al. Anal. Biochem.* **2007**, *367*, 152.

[3] BMG Labtech “How to optimise fluorescence gain”