ZEDI*XCITE* FLUOROGENIC MTG-ASSAY KIT

F015

Isopeptidase-activity based MTG assay (330/418 nm)

For *in vitro* research use only

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Intended use

Determination of MICROBIAL TRANSGLUTAMINASE (MTG) activity, specific and sensitive. Suitable for measurements of 2.5 nM to 80 nM (0.1 µg/mL to 3 µg/mL) of MTG.

Assay principle

MICROBIAL TRANSGLUTAMINASE (MTG) cleaves a dark quenching molecule from the side chain of MTG specific peptide A167 incorporating glycine methyl ester. Subsequently, the fluorescence of an N-terminal coupled dye increases and can be monitored (excitation wavelength 330 nm; emission wavelength 418 nm).

Test sample

Microbial Transglutaminase (MTG).

Reagent preparation, storage, and stability

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

Dissolve each vial of BUFFER REAGENT and DILUTION BUFFER in 18 mL of deionised water and mix carefully. Reconstituted buffer solutions can be stored at -20°C for several weeks.

Add 20 µl of SUBSTRATE REAGENT (1) to the reconstituted BUFFER REAGENT (2) and mix thoroughly (REAGENT MIXTURE, **RM**).

SAMPLE: If applicable, dissolve one vial of MICROBIAL TRANSGLUTAMINASE (MTG) as indicated in the respective CoA. Serial dilution from 800 nM to 12.5 nM (30.8 µg/mL to 0.5 µg/mL) is recommended. Final assay concentration will be one-tenth (compared to fig. 1, page 2). Other MTG samples should be diluted to the aforesaid range of concentration.

The prepared reagents must be used within 2 hours. Protect the REAGENT MIXTURE from light.

Procedure and Equipment

Prewarm the REAGENT MIXTURE for 10 minutes to 37°C before testing. Add REAGENT MIXTURE and SAMPLE as described below depending on your assay format:

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

Microtiter plate (96 well, 300 µL): 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. We recommend the measurement of SAMPLE in duplicate or triplicate.

Start the kinetic measurement at 37°C three minutes after starting the reaction using the following instrument parameters:

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

The REFERENCE LOT 2921aT001 (optional) is intended to be used for validating the kit in different laboratories and experimental settings to facilitate comparability of data. Use 30 µL of MTG-dilution (100 µL in case of cuvette measurement) in your assay.

Reagents in the kit

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

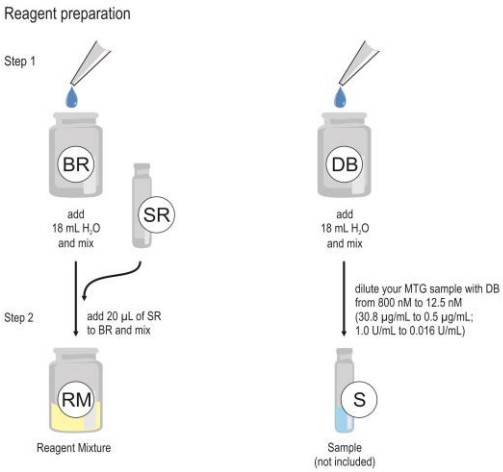
(2) BUFFER REAGENT (**BR**): 2 x 18 mL TRIS buffer pH 6.0 containing glycine methyl ester (lyophilizate).

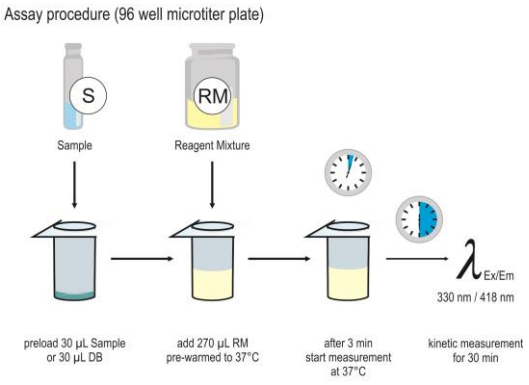
(3) DILUTION BUFFER (**DB**): 1 x 18 mL TRIS buffer pH 6.0 (lyophilizate).

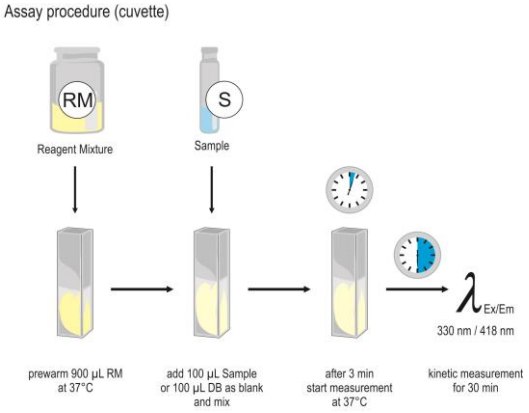
MICROBIAL TRANSGLUTAMINASE (MTG, T001, Zedira) - not included.

REFERENCE LOT 2921aT001 available (Zedira).

Schematic assay overview







Number of sample measurements

Using the cuvette method, the assay reagents per kit are sufficient for 38 measurements.

Using the microtiter plate method, the assay reagents per kit are sufficient for 130 measurements.

Results

The increase in fluorescence is proportional (2nd polynomial fit) to the MICROBIAL TRANSGLUTAMINASE (MTG) activity. The results can be evaluated using a reference curve. The data can be compared to figures 1a-c showing typical plots.

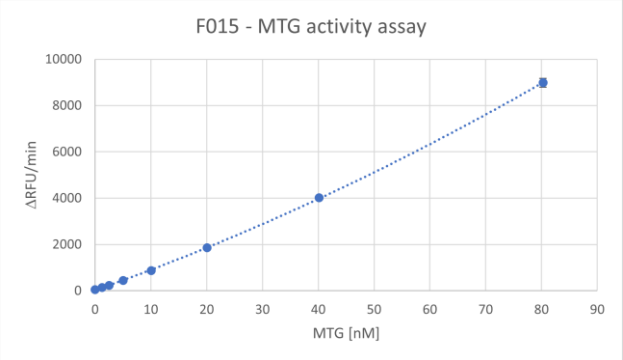


Fig. 1a

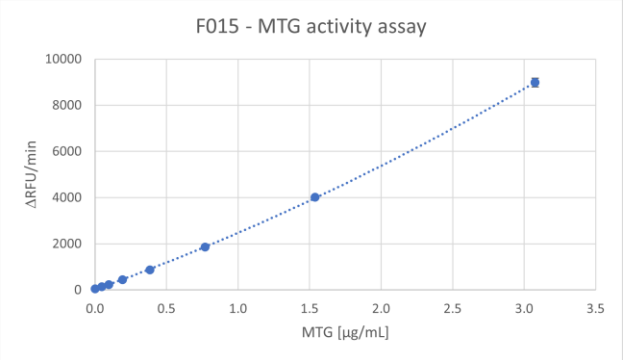


Fig. 1b

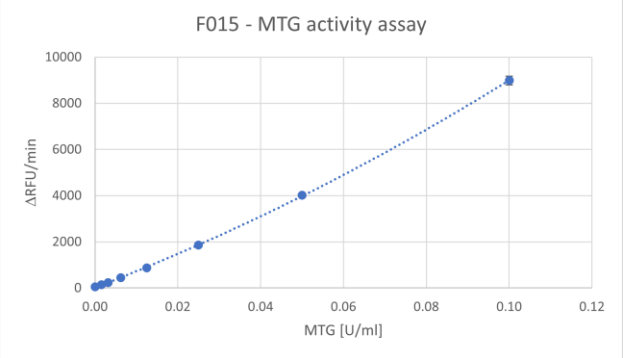


Fig. 1c

Fig. 1a-c: 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).
(One unit will catalyse 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))

Reference Range

The ZEDI*XCITE* FLUOROGENIC MTG-ASSAY (F015) is suitable for measurements of 2.5 nM to 80 nM (0.1 µg/mL to 3 µg/mL, 0.003 to 0.1 U/mL) of MTG.

Limitations

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

Precision

The coefficient of variation in the series was 3.5% for 80 nM (3.1 µg/mL, 0.1 U/mL) 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, 0.0031 U/mL).

References

1) Parameswaran, K.N. *et al. J. Biol. Chem.* **1997**, *272*, 10311.
2) Oertel, K. *et al. Anal. Biochem.* **2007**, *367*, 152.