MICROBIAL TRANSGLUTAMINASE ASSAY KIT

Art. No. Z009

Assay for the determination of microbial transglutaminase activity

For *in vitro* research use only

Zedira GmbH
Rösslerstraße 83
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Germany

Revision: 23.08.2024 | RN6.1

Intended use

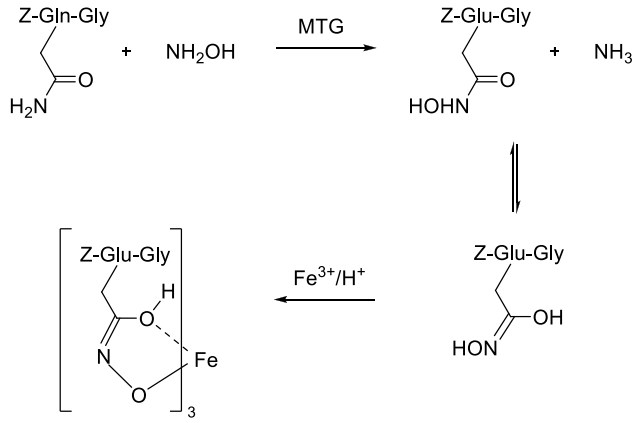
Determination of microbial transglutaminase activity (MTG).
Kit is sufficient for 3 x 11 measurements in cuvettes.
Measurement on MTP plates is possible too. See page 3.

Description

Transglutaminases are a family of enzymes that catalyse the posttranslational modification of proteins by inserting an isopeptide bond within or between polypeptide chains (Folk, J. E. and Cole, P. W., 1966). These enzymes catalyse the acyl transfer reaction between the γ-carboxyamide group of peptide-bound glutamine residues and a variety of primary amines, particularly the ε-amino group of lysine (Lorand L. et al., 1962). The resulting cross link is of great significance, since it is highly stable and also resistant to mechanical and proteolytic degradation.

Assay principle

The MICROBIAL TRANSGLUTAMINASE ASSAY KIT uses Z-Gln-Gly as the amine acceptor substrate and hydroxylamine as amine donor. In the presence of MTG hydroxylamine is incorporated into Z-Gln-Gly to form Z-glutamyl-hydroxamate-glycine which develops a colored complex with iron (III) detectable at 525 nm. The reaction is performed at pH 6.0.



Reagents in the kit

(A1) ACTIVITY REAGENT 1: lyophilized TRIS buffer containing Z-Gln-Gly and reduced glutathione, 3 vials
(A2) ACTIVITY REAGENT 2: Hydroxylamine, 3 vials
(S) STOP REAGENT: Hydrochloric acid [4% v/v], Iron (III) chloride, 3 vials
(P1) MICROBIAL TRANSGLUTAMINASE (positive control): 3 vials

Wear eye protection and suitable gloves when working with hydrochloric acid!

Storage and stability

P1 has to be stored at -20°C (shipment is possible at 4 – 8°C).
A1, A2, S has to be stored at 4 – 8°C. The unopened reagents are at least stable until the expiration date printed on the box.

Limitations

Please note that the assay is meant for research and development only.

Reagent preparation

Dissolve one vial of ACTIVITY REAGENT 1 (A1) in 5 ml of deionised water.
Dissolve one vial of ACTIVITY REAGENT 2 (A2) in 1 ml of deionised water.
Add 950 µL of the dissolved ACTIVITY REAGENT 2 (A2) to the vial of ACTIVITY REAGENT 1 (A1) and mix thoroughly. In the following, the resulting mixture is referred as ACTIVITY REAGENT.
STOP REAGENT (S) is ready to use.
Dissolve one vial of MICROBIAL TRANSGLUTAMINASES (P1) in 200 µL of deionised water. Use prepared reagents within 4 hours.

Equipment

The MICROBIAL TRANSGLUTAMINASE ASSAY KIT can be used in standard spectrophotometers with polystyrene 1 mL cuvettes. Refer to the instructions of the manufacturer.

Assay procedure: cuvettes

Prewarm for each sample 500 µL of ACTIVITY REAGENT for 10 minutes in suitable reaction tubes (1.5 mL) to 37°C before testing. Start the reaction by adding 50 µL of sample into 500 µL of ACTIVITY REAGENT. Use 50 µL of deionised water or buffer instead of sample to generate a blank. Stop the reaction exactly after 10 minutes by adding 500 µL of STOP REAGENT (S). Use a centrifuge to separate precipitations for 5 minutes, 10,000 x g at ambient temperature. The reaction mixture (1 mL) can be transferred to a suitable cuvette and measured at 525 nm. The MICROBIAL TRANSGLUTAMINASE (P1) is intended to be used for validating the efficacy and quality of the kit device. Use 50 µL of MICROBIAL TRANSGLUTAMINASE (P1) to obtain a volumetric activity of > 0.8 U/mL ($\Delta E_{525\,nm}$ > 0.33). For reliable results $\Delta E_{525\,nm}$ of the samples should always be in the range from 0.1 to 0.9.
See also a schematic assay overview on the next page.

Results

One unit of microbial transglutaminase activity is defined as the amount of enzyme, which causes the formation of 1.0 µmole of hydroxamate per minute by catalysing the reaction between Z-Gln-Gly and hydroxylamine at pH 6.0 at 37ºC (Folk and Cole, 1966).

The activity can be calculated using following equation.

$$Activity\ \left[\frac{U}{mL}\right] = \left[\frac{\Delta E \times V}{\varepsilon \times d \times v \times t}\right] = \Delta E \times 2.64\ \left[\frac{\mu mol}{min \times mL}\right]$$

With: ΔE = extinction (525 nm), V= total volume (1.050 mL), d = path length cuvette (1 cm), t = time 10 min), v = sample volume (50 µL), ε = 0.795 mL x µmol^{-1} x cm^{-1} (note: molar extinction coefficient of glutamyl-hydroxamate in the stopped assay solution differs from the value obtained using the protocol by Folk and Cole, 1966)

References

(1) Lorand L., Konishi K., Jacobsen A., Nature 1962;194:1148-1149.
(2) Grossowicz N, Wainfan E, Borek E, Waelsch H., J Biol Chem. 1950 Nov;187(1):111-25.
(3) Folk, J. E. und Cole, P. W., Biochim. Biophys. Acta 1966, 122, 244-64.
(4) Rickert et al., Protein Sci. 2015, accepted manuscript (DOI: 10.1002/pro.2833).

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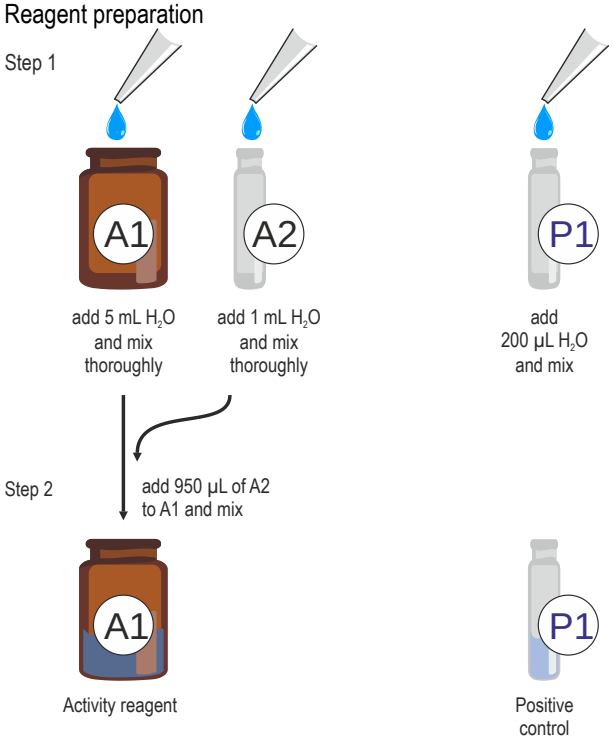
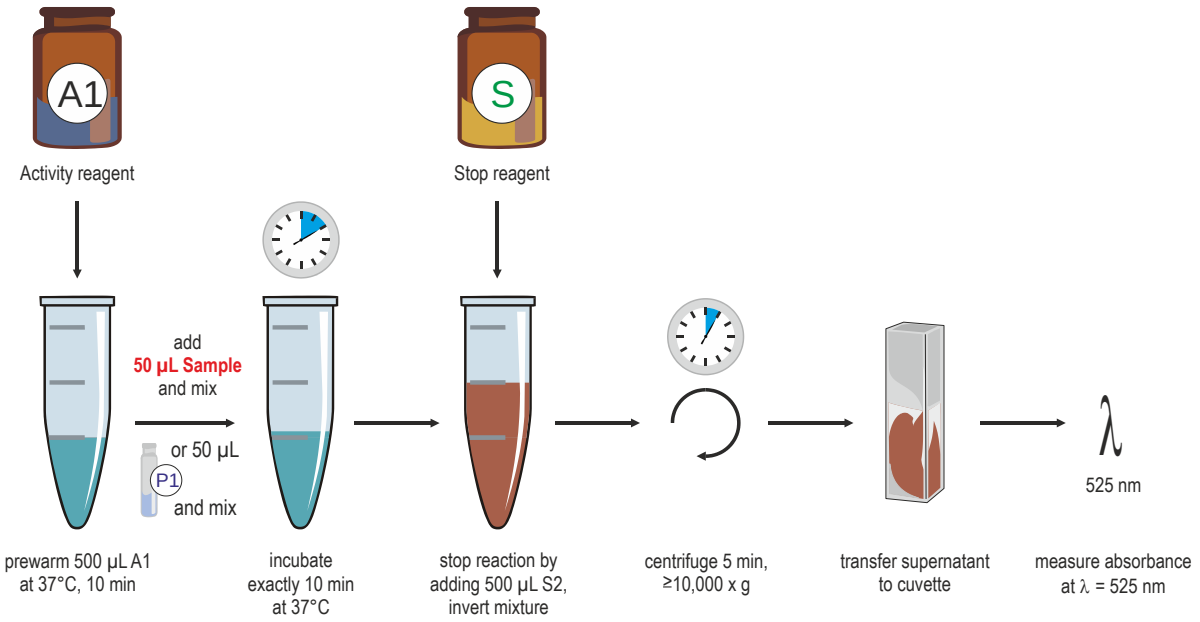
Schematic assay overview

Reagent preparation



Watch the video on YouTube!

Assay procedure in cuvettes



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Assay procedure: microtiter plates

Determination of transglutaminase activity in microtiter plates can be performed at ambient temperature (~23°C) or at 37°C if constant temperature can be ensured.

Preload your plate with 10 µL of sample or POSITIVE CONTROL (P1). Use 10 µL of deionised water or buffer instead of sample to generate a blank.
Add 150 µL of ACTIVITY REAGENT to start the reaction. Note: Use pre-warmed ACTIVITY REAGENT for reaction conditions at 37°C.
Stop the reaction exactly after 10 minutes by adding 140 µL of STOP REAGENT (S).
For reliable results $\Delta E_{525\ nm}$ of the samples should always be in the range from 0.2 to 0.9.

The activity can be calculated using following equation.

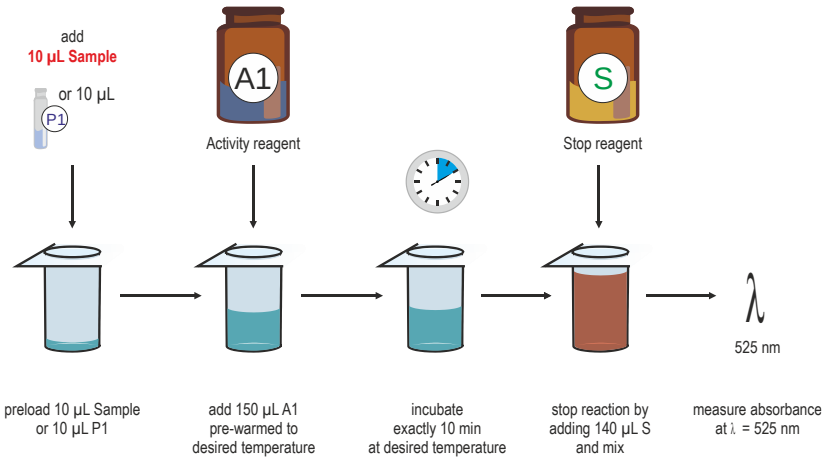
$$Activity\ \left[\frac{U}{mL}\right] = \left[\frac{\Delta E \times V}{\varepsilon \times d \times v \times t}\right] = \Delta E \times 5.61\ \left[\frac{\mu mol}{min \times mL}\right]$$

With: ΔE = extinction (525 nm), V = total volume (0.3 mL),
d = path length MTP (0.82 cm), t = time (10 min), v = sample volume (10 µL), ε = 0.652 mL x µmol^{-1} x cm^{-1}

Calibration

We recommend the calibration of the assay on your device by determining the molar extinction coefficient using Z-Glutamyl(γ-hydroxamate)-glycine (Zedira product code Z018).

Assay procedure (96 well microtiter plate)



Microbial Transglutaminase Assay may be performed at ambient temperature.

PLEASE NOTE:

- Reaction at ambient temperature results in lower MTG activity and loss of assay sensitivity.
- Activities measured at different temperatures cannot be compared.
- Pre-warm ACTIVITY REAGENT to desired reaction condition (e.g. 37°C)