TISSUE TRANSGLUTAMINASE ASSAY KIT

Art. No. Z010

Assay for the determination of tissue transglutaminase activity

For *in vitro* research use only

Zedira GmbH
Rösslerstraße 83
64293 Darmstadt
Germany

Revision: 26/01/2017 | RN1.0

Intended use

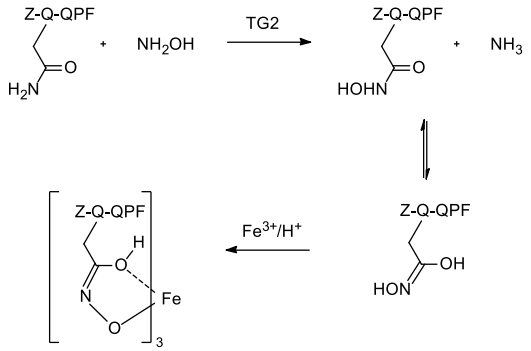
Determination of tissue transglutaminase activity (tTG, TG2). Kit is sufficient for 3 x 11 measurements in cuvettes or 3 x 36 measurements in 96 well microtiter plates (MTP).

Introduction

TG2 is present in various tissues and involved in a plentitude of physiological as well as pathological processes. The enzyme catalyses 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). This assay enables the measurement of TG2 activity according to the chromogenic hydroxamate detection principle (Grossowicz, N. et al., 1950).

Assay principle

The TISSUE TRANSGLUTAMINASE ASSAY KIT uses Z-QQPF as the amine acceptor substrate and hydroxylamine as amine donor. In the presence of tTG hydoxylamine is incorporated into Z-QQPF to form Z-glutamyl-hydroxamate-QPF which develops a colored complex with iron (III) detectable at 525 nm.



Reagents in the kit

(1A) ACTIVITY REAGENT 1A: lyophilized MOPS buffer pH 7.6 containing Z-QQPF, DTT and calcium, 3 vials
(2A) ACTIVITY REAGENT 2A: Hydroxylamine, 3 vials
(3S) STOP REAGENT 3S: Hydrochloric acid [4% v/v], Iron (III) chloride, 3 vials
(4P) TG2 POSITIVE CONTROL 4P: lyophilized TG2, 3 vials

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

Storage and stability

4P has to be stored at -20°C (shipment is possible at 4 – 8°C).
1A, 2A, 3S has to be stored at 4 – 8°C. The unopened reagents are at least stable until the expiration date printed on the box.

Reagent preparation

Dissolve one vial of ACTIVITY REAGENT 1A (1A) in 5 ml of deionised water. Dissolve one vial of ACTIVITY REAGENT 2A (2A) in 1 ml of deionised water. Add 950 µL of the dissolved ACTIVITY REAGENT 2A (2A) to the vial of ACTIVITY REAGENT 1A (1A) and mix thoroughly. In the following, the resulting mixture is referred as ACTIVITY REAGENT.
STOP REAGENT (3S) is ready to use.
Dissolve one vial of TG2 POSITIVE CONTROL (4P) in 300 µL of deionised water. Use prepared reagents within 4 hours.

Limitations

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

Equipment

The TISSUE TRANSGLUTAMINASE ASSAY KIT can be used in standard spectrophotometers with polystyrene 1 mL cuvettes or MTP.
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 (3S). Use a centrifuge to separate precipitates for 5 min, 10,000 x g at ambient temperature. The reaction mixture (1 mL) can be transferred to a suitable cuvette and measured at 525 nm. TG2 POSITIVE CONTROL (4P) is intended to be used for validating the efficacy and quality of the kit device. Use 50 µL of TG2 POSITIVE CONTROL (4P) to maintain a volumetric activity of > 1.6 U/mL ($\Delta E_{525\ nm}$ > 0.58).
For reliable results $\Delta E_{525\ nm}$ of the samples should always be in the range from 0.1 to 0.8.
See also the schematic assay overview on the next page.

Results

One unit of tissue transglutaminase activity is defined as the amount of enzyme, which causes the formation of 1.0 µmole of Z-glutamyl-hydroxamate-QPF per minute by catalysing the reaction between Z-QQPF and hydroxylamine at pH 7.6 at 37ºC.

The activity can be calculated using following equation.

$$Activity\left[\frac{U}{mL}\right]=\left[\frac{\Delta E\times V}{\varepsilon\times d\times v\times t}\right]=\Delta E\times 2.77\left[\frac{\mu mol}{\min\times mL}\right]$$

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 = 0.757 mL x µmol^{-1} x cm^{-1}

Assay may be performed at ambient temperature.

PLEASE NOTE: Reaction at ambient temperature results in lower activity and loss of sensitivity. Activity measured at different temperatures cannot be compared.

Assay procedure: MTP (microtiter plates)

Determination of TG2 activity in microtiter plates is recommended at ambient temperature (23°C). Preload your plate with 150 µL of ACTIVITY REAGENT. Start the reaction by adding 10 µL of sample or TG2 POSITIVE CONTROL (4P). Use 10 µL of deionised water or buffer instead of sample to generate a blank. Stop the reaction exactly after 10 minutes by adding 140 µL of STOP REAGENT (3S).

The activity can be calculated using following equation.

$$Activity\left[\frac{U}{mL}\right]=\left[\frac{\Delta E\times V}{\varepsilon\times d\times v\times t}\right]=\Delta E\times 5.29\left[\frac{\mu mol}{\min\times mL}\right]$$

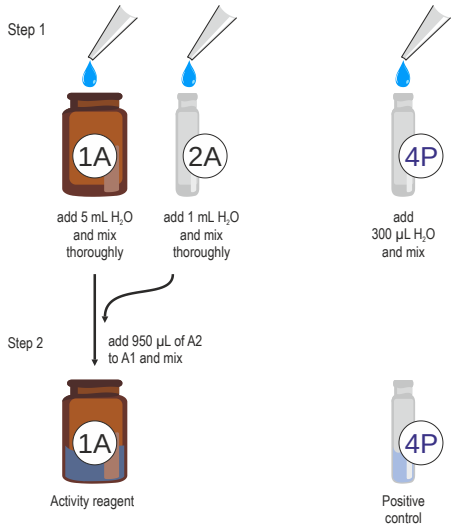
With: ΔE = extinction (525 nm), V= total volume (0.3 mL),
d = path length MTP (0.82 cm), t = time (10 min), v = sample volume (10 µL), ε = 0.692 mL x µmol^{-1} x cm^{-1}

Zedira GmbH
Rösslerstraße 83
64293 Darmstadt
Germany

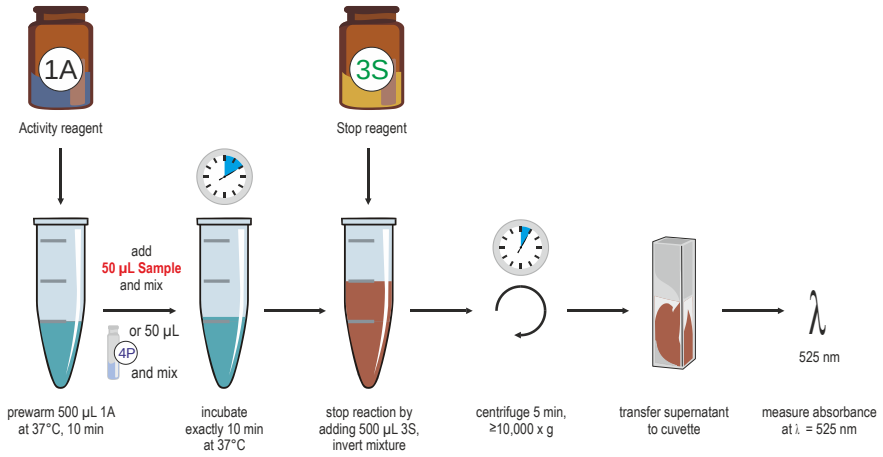
Revision: 26/01/2017 | RN1.0

Schematic assay overview

Reagent preparation



Assay procedure (Cuvettes)



Assay procedure (96 well microtiter plate)