

TISSUE TRANSGLUTAMINASE ASSAY KIT, FLUORESCENT

REF F002
Assay for the determination of TG2 activity

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For *in vitro* research use only

RN 2.0
Revision: 07/07/2014

Intended use

Determination of tissue transglutaminase (TG2) activity.

Assay principle

Tissue transglutaminase (TG2) cleaves a dark quenching molecule from the side chain of a modified peptide incorporating glycine methyl ester. Subsequently, the fluorescence of an N-terminal coupled dye increases and can be monitored on-line (excitation wavelength 313 nm; emission wavelength 418 nm). The modified peptide used is intellectual property of Zedira[®].

Test sample

Recombinant human tissue transglutaminase (hTG2).

Reagents in the kit

- (1) SUBSTRATE REAGENT: 2 x 25 µl modified peptide dissolved in DMSO
- (2) BUFFER REAGENT: 2 x 20 ml TRIS buffer pH 7.5 containing calcium chloride, sodium chloride, glycine methyl ester, glutathion.
- (3) DILUTION BUFFER: 1 x 20 ml TRIS buffer pH 7.5 containing calcium chloride and sodium chloride

Reagent preparation, storage and stability

In their original packing box, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

Add 20 µl of SUBSTRATE REAGENT (1) to one vial of BUFFER REAGENT (2) and mix thoroughly (REAGENT MIXTURE). We recommend rinsing the vial of SUBSTRATE REAGENT several times with the REAGENT MIXTURE to ensure a quantitative transfer.

Stability of the REAGENT MIXTURE after reconstitution

Temperature	Stability
37 °C	4 hours
20 °C	8 hours
4 °C	1 day
-20 °C	2 months

Protect the REAGENT MIXTURE from light!

Equipment

The Fluorescent TG2 assay can be used in standard fluorescence spectrophotometers. Refer to the instructions of the manufacturer.

Procedure

Prewarm the REAGENT MIXTURE in suitable fluorescence cuvettes to 37°C before testing. Add 100 µl of sample to 900 µl of REAGENT MIXTURE, mix thoroughly and start measuring at 37 °C immediately at the following instrument parameters:

Excitation wavelength	313 nm
Emission wavelength	418 nm
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)

However, these instrument parameters are recommendations only and should be adjusted to the instrument used to obtain a suitable signal to noise ratio.

Results

The increase in fluorescence is proportional to the tissue transglutaminase activity. The results can be evaluated using a reference curve. To calculate the reference curve, TG2 standard is diluted with DILUTION BUFFER (3) and measured in double determination. The data can be compared to figure 1 showing a typical standard curve.

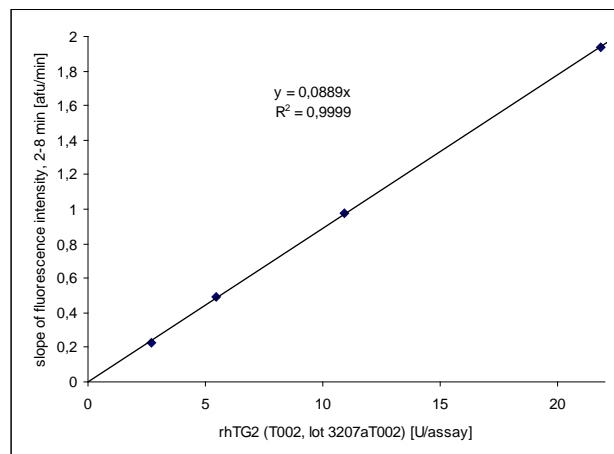


Fig. 1: Reference curve for recombinant human tissue transglutaminase (hTG2) diluted with DILUTION BUFFER (3) and measured in double determination.

Linearity

The fluorescent TG2 Assay (F002) is suitable for measurements of up to 20.0 U TG2 per 100 µl.

Limitations

The FLUORESCENT TG2-ASSAY (F002) is meant for research and development only.

Remarks

This assay was developed and optimised for purified TG2. Using undefined probes, e.g. cell homogenate, the assay can be seriously influenced. This is probably due to e.g. proteases, which cleave the peptidic backbone of the substrate, resulting in increased fluorescence independently of TG2 activity. Therefore we recommend to perform suitable controls e.g. by inhibition of TG2 using suitable inhibitors.

References

- 1) Oertel K and Pasternack R: Fluorescence-based kinetic determination of the activity of Transglutaminases (EP04019090.2: US60/603,374).

Verwendete Symbole / Used symbols / Använda symboler / Anvendte symboler

In-vitro-Diagnostikum In vitro diagnostic medical device In vitro diagnostisk udstyr In vitro diagnostisk medicinsk produkt	Artikel-Nummer Article number Varenummer Artikel nummer	Chargen-Bezeichnung Lot code Batch nr. Tilvekningskod	Verwendbar bis Use by Anvendelse Anvendelse	Lagerungstemperatur Storage temperature Temperatur begrensninger Temperatur græns	Hergestellt von Manufacturer Producent Tilverkare	Biologisches Risiko Biological risks Miljø oplysninger Biologiska risker	Gebrauchsinformation beachten Consult instructions for use Se vejledning for anvendelse Ta del av instruktionerna före användning
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