MTG-ANiTA-KIT

REF M001

Ammonium-**N**icotinamidADPH-GLDH-**T**ransglutaminase **A**ssay

For in vitro research use only

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Intended use

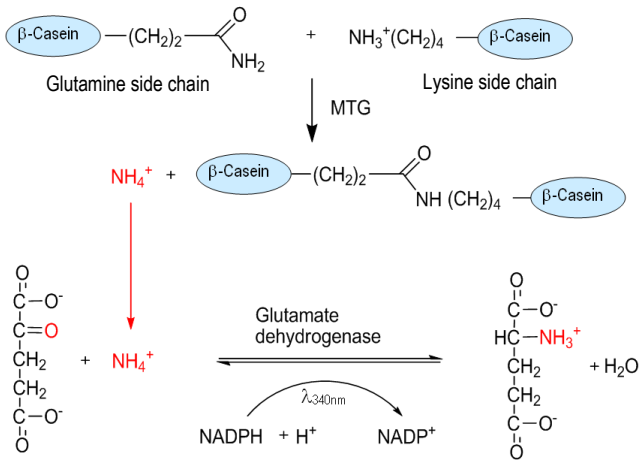
Determination of microbial transglutaminase activity (MTG).
Kit is sufficient for 2 x 11 measurements.

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 MTG-ANiTA-KIT uses β-Casein as high molecular weight substrate. Transglutaminase catalyzes the formation of isopeptide bonds between protein-bound glutamine and lysine releasing one ammonium molecule per isopeptide bond formed.
In the indicator reaction the amount of released ammonium produced by transglutaminase is monitored in a glutamate dehydrogenase (GLDH) catalysed NADPH-dependent reaction. The consumption of NADPH is measured spectrophotometrically by the decrease of absorbance at 340 nm.



Kit contents

BD-BUFFER, 2 vials
[GLDH, ADP, α-KETOGLUTARATE]-MIX, 2 vials
NADPH (AD-REAGENT), 2 vials
β-Casein (TR-BUFFER), 2 vials
MTG-BLOCKER (INHIBITOR REAGENT), 2 vials

Storage and stability

MTG-ANiTA-KIT should be stored at 4 – 8°C. The unopened reagents are stable until the expiration date printed on the box.

Equipment

The MTG-ANiTA-KIT can be used in standard spectrophotometers with UV-cuvettes (e.g. BRAND Cat. No.:7591 50, semi-micro). Refer to the instructions of the manufacturer.

Reagent preparation

Remove the kit components from cooling conditions to ambient temperature 1 h prior to use.
Add 6 mL of BD-BUFFER to the β-Casein (TR-BUFFER)-vial, in order to dissolve β-Casein and allow standing for 20 min at ambient temperature. Shake gently until solid dissolves. Do not vortex.
Add 5.5 mL BD-BUFFER to the NADPH-vial.
Dissolve [GLDH, ADP, α-KETOGLUTARATE]-MIX in 600 µL of BD-BUFFER and transfer 500 µL into the 5.5 mL NADPH preparation to obtain Ammonium Detection Reagent (AD-REAGENT).
Transfer 1 mL of BD-BUFFER into one vial of MTG-BLOCKER to obtain the INHIBITOR REAGENT. Shake vigorously until solid dissolves.
Store the AD-REAGENT, TR-BUFFER and the INHIBITOR REAGENT at ambient temperature and use it after reconstitution within 2 hours.

Assay procedure

Dispensation of TR-BUFFER and AD-REAGENT by use of an electronic dispenser (e.g. Eppendorf 4986 000.017) are highly recommended.
Add 500 µL of TR-BUFFER slowly in a suitable reaction tubes (e.g. Eppendorf LoBind 1.5 mL) and preheat for 10 min at 37°C.
Start the reaction by adding 50 µl of sample into the preheated TR-BUFFER and mix well by pipetting 5 times up and down. Use 50 µl of BD-BUFFER instead of sample to generate a blank. Stop the reaction exactly after 10 minutes by adding 50 µL of INHIBITOR REAGENT and mix well by pipetting 5 times up and down.
Add 500 µL of AD-REAGENT to the stopped reaction mixture and incubate 30 min at 37°C.
Vortex and transfer the reaction mixture to a suitable UV-cuvette and measure the consumption of NADPH corresponding to produced ammonium at 340 nm.
A schematic assay overview is given on the next page. For reliable results $\Delta E_{340\,nm}$ of the samples after 30 min should always be in the range from 0.3 to 1.2.

Results

One unit of transglutaminase activity is defined as the amount of enzyme, which catalyses the formation of 1.0 µmole of ammonium per minute.
The results can be evaluated using following equation.

$$Activity\left[\frac{U}{mL}\right]=\left[\frac{\Delta E_{340nm}\times F_{AD}\times V_{TR}}{\varepsilon\times d\times v\times t}\right]=\Delta E_{340nm}\times f\times 0.3452\left[\frac{\mu mol}{\min\times mL}\right]$$

With:

ΔE_{340nm} = $\Delta E_{340\,nm}$ (blank) - $\Delta E_{340\,nm}$ (sample)
V_{TR} = transglutaminase assay volume (0.6 mL),
d = cuvette diameter (1 cm)
t = time (10 min)
v = sample volume (0.05 mL),
ε (NADPH) = 6.3735 mL x µmol^{-1} x cm^{-1}
F_{AD} = dilution factor by ammonium detection (1.8333)
f = sample dilution factor

Limitations

It should be noted that the assay is meant for research and development only.

References

(1) Lorand L., Konishi K., Jacobsen A., Nature 1962;194:1148-1149.
(2) Folk, J. E. und Cole, P. W., Biochim. Biophys. Acta 1966, 122, 244-64.

Verwendete Symbole / Used symbols

IVD	REF	LOT				
vitro-Diagnostikum In vitro diagnostic medical device	Artikel-Nummer Article number	Chargen-Bezeichnung Lot code	Verwendbar bis Use by	Lagerungstemperatur Storage temperature	Hergestellt von Manufacturer	Gebrauchsinformation beachten Consult instructions for use

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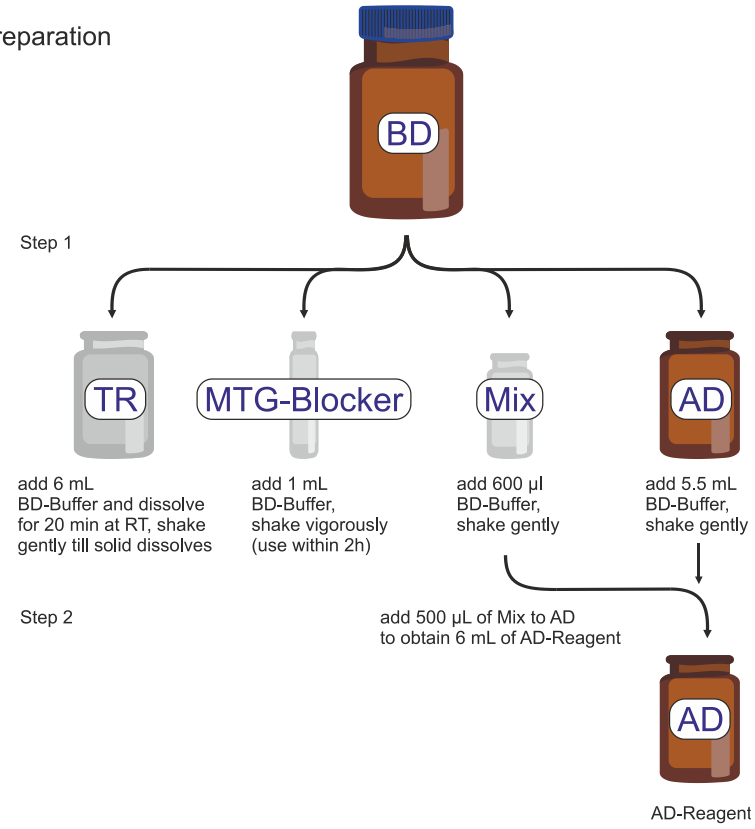
Revision flow diagram: 10/12/2014

Schematic assay overview

Reagent preparation



Assay procedure