Product Information & ELISA Manual

Transglutaminase 2/TGM2 Assay Kit (Colorimetric)

NBP1-37008

Enzyme-linked Immunosorbent Assay
for quantitative detection.

Contact

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Introduction

Transglutaminases (EC. 2.3.213, R- glutamyl-peptide amine γ-glutamyl-transferase) are a family of calcium dependent enzymes which catalyse an acyl transferase reaction between the γ-carboxamide group of peptide bound glutamine and various primary amines.

The ε-amino group of lysine residues as well as some polyamines are the physiological amine donors, but some non-physiological amines can also be used by the enzyme. In vertebrates, transglutaminases (TGs) are widely distributed in various organs, tissues and body fluids. TGases are involved in a variety of roles including blood clotting, formation of the cornified envelope of the epidermis and its appendages (hair, nail, callus), stabilization of intra and extracellular matrices and cross-linking of cell envelopes in apoptosis. Among the various strategies developed to measure TGase activity, the most sensitive and accurate quantification methods have been the radioactive and fluorimetric assays that detect the incorporation of labeled molecules, such as [^{3}H/^{14}C]-putrescine or, monodansyl-cadaverine respectively, into glutamyl substrates such as casein or synthetic peptides.

Solid phase assays developed so far in these principles have been compromise by a high background signal, low sensitivity compare to radiolabeling methods and the lack of the specificity. This kit overcomes these problems and constitutes a highly sensitive and specific TG2 solid phase microassay

The kit is designed to have the following advantages:

- **Measurement of tissue Transglutaminase (tTG/TG2) with low or no interference with other TGs isoforms.**
- **High sensitivity (equivalent to commonly used radiometric filter paper method).**
- **Rapid test (performance time is 45 min).**
- **Simultaneous screening using 8-96 well strips.**

Principle of the assay

The Transglutaminase 2/TGM2 Assay Kit (Colorimetric) uses biotinylated T26 peptide (Biotin-pepT26) as the first substrate (amine- acceptor/acyl-donor) and an amine-donor/acyl-acceptor as a second substrate.

Samples suspected of containing TG2 are incubated with calcium, dithiothreitol (DTT) and Biotin-pepT26 in the wells of microtiter plates containing the amine substrate. In the presence of TG2, the γ carboxamide of the glutaminyl residue of the biotin-pepT26 is incorporated into the amine substrate to form biotinylated isopetide bound.

The system is coupled to Streptavidin labelled peroxidase (SAv-HRP). SAv-HRP is revealed using H_2O_2 as HRP substrate and tetramethyl benzidine as electron acceptor (chromogen).

Kit Components

Plates and Reagents	Quantity
Microtiter strips coated with amine substrate	12x8 strips microtiter wells
DTT	0.2 mL
EDTA (Negative Control)	1 mL
Reaction Buffer (Biotin-pepT26/CaCl2)	2 vials (lyophilized powder)
Enzyme Tracer (SAv-HRP)	50 µL
Wash Buffer 10X	30 mL
Diluent Buffer 10X	10 mL
HRP Substrate RTU (ready To use)	12 mL
Blocking Reagent	12 mL
opr0036 Positive Control: Recombinant human TG2 (rhTG2)	2 vials

Storage and Stability

The kit is shipped on ice and storage at +4°C is recommended. Upon arrival, the DTT, Enzyme Tracer (SAv-HRP), and opr0036 Positive Control should be stored at –20°C. All the other components of the kit should be kept at +4°C.

Assay procedure

PRELIMINARY OPERATIONS

1. Identify a sufficient number of wells/strips to run in duplicate according to the reaction scheme (see below):
    a) blank
    b) samples
    c) negative control

    (Controls and samples should all be subjected to exactly the same assay procedure.)

2. Wash Buffer 10X and Diluent Buffer 10X should be diluted 1/10 with ultrapure water to obtain a 1X solution, respectively.

3. Reconstitute the Reaction Buffer (lyophilized powder) in 3 mL of ultrapure water. Place on ice until use, or storage at -20° C.
    Note that: the concentration of CaCl2 in reconstituted Reaction Buffer is 11,6 mM.

4. Prepare the samples at a suitable concentration using the Diluent Buffer 1X. Samples from cells or tissues should be centrifuged before use.

    Note that: For cell lysis, use TBS +1% Triton + protease inhibitor cocktail as lysis buffer. Lysis buffer should NOT contain EDTA, EGTA, SED, Thimerosal or any reagent that would degrade proteins or inhibit biological activity of the TG2.

5. Prepare 20-30 ml of a solution of NaOH 0,1M

6. Prepare 20-30 ml of a solution of BSA 0.5% in Wash Buffer 1X (0.1g / 20ml)

7. Prepare a sufficient volume of **Assay Mixture**:

    For 1 x 8 strips microtiter wells: Add 10 µL of DTT per 0,5 mL of reconstituted Reaction Buffer and place on ice until use. Of note, unused Assay Mixture should be discarded.

REACTION SCHEME

	Sample / Enzyme	Dilution Buffer	EDTA	Assay Mixture	Final volume
Blank	-	60 µL	-	50 µL	110 µL
Test	x*µL	60 – x*µL	-	50 µL	110 µL
Standard Curve	50 µL	10 µL	-	50 µL	110 µL
Negative Control	50 µL	-	10 µL	50 µL	110 µL

x*: Volume of test sample added to the reaction

PERFORM THE Transglutaminase 2/TGM2 Assay AS FOLLOWS

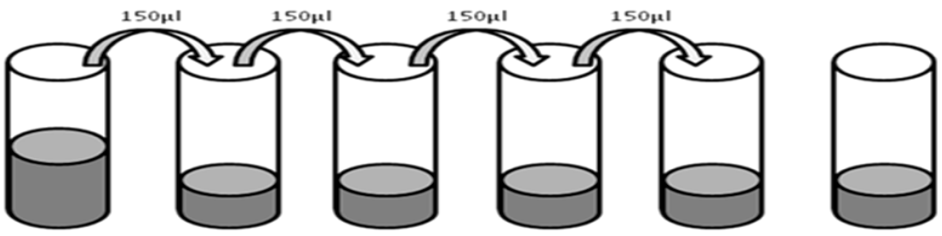
1. Dispense 150 µL per well of **Wash Buffer 1X**. Incubate 15 minutes at 37°C.
2. Remove the Wash Buffer 1X from the strips.
3. Dispense 50 µL per well of ice cold **Assay Mixture**.
4. Add 10 µL of **EDTA** for the Negative Control and mix well by pipetting up and down.
5. According to the reaction scheme, add in the corresponding wells, 50 to 60 µL of ice cold **Sample/Enzyme**.
6. Incubate for 15-30 minutes at 37°C and shake gently.
7. During incubation, dilute the **SAv-HRP** at 1/2 000 in "**Wash Buffer 1X**" and mix gently by inversion. Place on ice until use.
8. Wash the wells once with 200 µL/well of **Wash Buffer 1X**.
9. Wash the wells once with 200 µL/well of **NaOH 0,1M**.
10. Wash the wells two times with 200 µL/well of **Wash Buffer 1X**.
11. Wash the wells once with 200µl/well of BSA 0.5%.
12. Dispense 100 µL of the freshly prepared **SAv-HRP** solution per well.
13. Incubate for 15 minutes at 37°C and shake gently.
14. Wash the wells three times with 200 µL/well of **Wash Buffer 1X**.
15. Dispense 100 µL per well of **HRP substrate**.
16. Incubate for 1 to 5 minutes at room temperature.
17. Dispense 100 µL per well of **Blocking Reagent**.
18. Measure the optical density of each well at 450 nm (OD450nm).

TG2 Standard curve

Planning of a standard curve using TG2 enzyme of known activity is recommended for each set of samples. For accurate results we recommend to duplicate the assays.

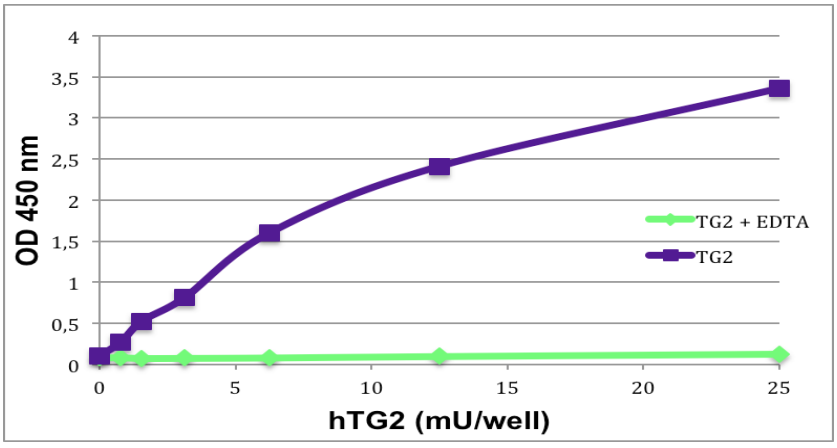
Preparation of positive control (lot dependent*):

- In the first tube : add qsp **150 mU** of rhTG2 (opr0036) in 300 µl of Diluent buffer 1X
- For the 5 other tubes : add 150 µl of Diluent buffer 1X into each tube.
- Use the first standard solution (first tube) to prepare the subsequent serial dilutions (see below). Mix each tube thoroughly before next transfer. The Diluent Buffer 1X serves as the blank in the curve.



*The exact quantity of opr0036 Positive control: Recombinant human TG2 (rhTG2) depends on the batch used and can be calculated with the data indicated on the lot specific COA. Contact technical support if you are unable to locate this information.

Example of results



This standard curve is provided for demonstration only (the units of TG2 in each tube is dependant of the Lot of opr0036 used).
A standard curve should be generated for each set of samples assayed.

Calculation of results

Planning of a standard curve using TG2 enzyme of known activity is recommended. Several experiments using human recombinant TG2 [Activity: see lot specific COA; one unit catalyse the formation of 1µmole of hydroxamate per minute from Z-Gln-Gly-OH and hydroxylamine at pH 6.0 at 37°C] have shown that 50 mU/mL correspond to an absorbance value (OD) of 1.4 $\pm$ 0.06 at 450 nm.

If other TG2 are used in this assay the reference value may be different. End users should establish their own set of reference values.

Inhibition reaction assay

The kit can be used to assay enzyme inhibitors. For performing an inhibition reaction, first optimize the concentration of the enzyme to be used.
After step 2 (see above), add 1-25 µL of Sample/Enzyme and 1-25 µL inhibitor of choice to the appropriate wells.
Adjust the volume to 50 µL with Diluent Buffer 1X. Pre-incubate the enzyme with the inhibitors for 1-5 minutes on ice.
Load 50 µL of Assay Mixture and 10 µL of EDTA for the Negative Control.
Continue from step 6.

Safety procedure

- The product is not licensed or approved for administration to humans or to animals.
- Standard Laboratory Practices should be followed when handling this material.
- **Handle with care HRP substrate and blocking reagent. These reagents are irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.**

References

1. Perez Alea M. and al. Development of an isoenzyme-specific colorimetric assay for tissue transglutaminase 2 cross-linking activity. *Anal . Biochem*., 2009; 289, 150-156.
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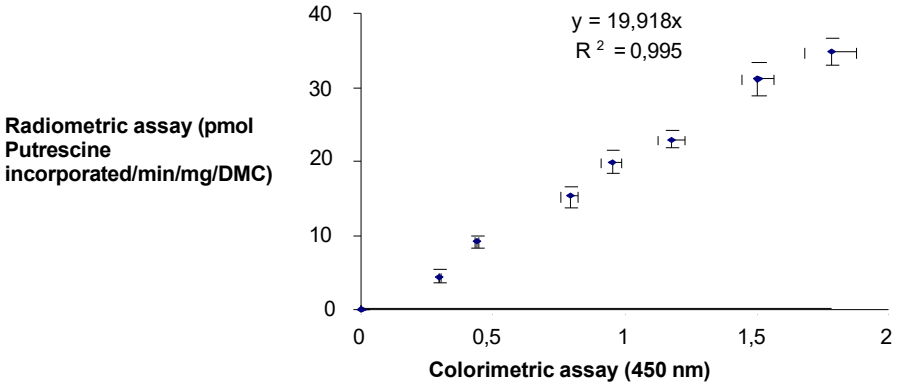
3. Folk JE and Finlayson JS. The ε(γ-glutamyl)lysine cross link and the catalytic role of transglutaminases. *Advances in Protein Chemistry*, 1977, 31: 1-33.

4. Lorand L and al, A filter paper assay for transamidating enzymes using radioactive amine substrate. *Anal . Biochem*., 1972; 50, 623-631.
5. Villalobos E. and al. Molecular cloning and characterization of a maize transglutaminase complementary DNA. *Gene 336*., 2004, 93-104.
6. Thomas V. and al. Development and evaluation of a modified colorimetric solid-phase microassay for measuring the activity of cellular and plasma (Factor XIII) transglutaminases *Biotechnol. Appl. Biochem. (2006) 43, 171–179.*
7. Sugimora Y. and al. Screening for the Preferred Substrate Sequence of Transglutaminase Using a Phage-displayed Peptide Library: identification of peptide substrates for tgase 2 and factor XIIIA. *J Biol. Chem*; 2006; 281, 17699–706

Examples of results

Ex. 1: Comparison of colorimetric and radiometric TG2 assays



Ex. 2: Assay specificity for TG2