Opr0001  Transglutaminase Colorimetric Microassay Kit

Introduction

Transglutaminases (EC. 2.3.2.13, 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 (TGases) 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.

All the enzymatic assays for TGases referred to above were carried out under optimal conditions of pH, Ca\(^{2+}\) and substrates with dimethylcasein as the first substrate and radiolabelled putrescine or dansyl cadaverine as the second substrate. Another approach to quantitating TGase activity has been to measure ammonia released during isopeptide bond formation. These techniques are time consuming and labor intensive.

To completely overcome these problem we have developed a three labor step solid phase micro assay : "TG-Covtest".

Principle of the assay

The TG-Covtest uses CBZ-Gln-Gly as the first substrate (amine acceptor) and biotin cadaverine as second (amine donor). In the 1st step, samples suspected of containing TGase are incubated with calcium, dithiothreitol (DTT) and biotinylated cadaverine in the wells of microtiter plates to which CBZ-Gln-Gly had been covalently coupled previously. In the presence of TGase biotinylated cadaverine is incorporated γ-carboamde of the glutaminyl residue of the dipeptide to form γ-glutamyl cadaverine biotin.

In the second step streptavidin-labelled peroxidase is added to the wells. In the third step, peroxidase activity is revealed using H\(_2\)O\(_2\) as HRP substrate and tetramethyl benzidine as electron acceptor (chromogen).

The TG-Covtest has two original features:
- The use of covalently coupled CBZ-Gln-Gly solves the problem of leaching out of absorbed substrate
- The second feature is that 96 samples can be simultaneously screened in 30 minutes time.

Reagents of the kit

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>R1 : Microtiter plates with covalently bound CBZ-Gln-Gly</td>
<td>3 x 96-well plate 1 mL</td>
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<tr>
<td>R2 : Negative control</td>
<td>3 vials (1vial per plate) 50 µL</td>
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<tr>
<td>R3 : Biotin-cadaverine/CaCl(_2) (lyophilized powder)</td>
<td>20 mL</td>
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<tr>
<td>R4 : Enzyme tracer</td>
<td>50 mL</td>
</tr>
<tr>
<td>R5 : Diluent buffer 10X</td>
<td>1,25 mL</td>
</tr>
<tr>
<td>R6 : HRP substrate</td>
<td>25 mL</td>
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<tr>
<td>R7 : Chromogen</td>
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<tr>
<td>R8 : Blocking reagent</td>
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</tbody>
</table>

Storage and Preparation of reagents

**Microtiter plates with covalently bound CBZ-Gln-Gly (R1)**

The plates should be kept at +4°C and are ready to use.

**Negative control (R2)**

The negative control should be kept at +4°C and is ready to use.

The negative control contains EDTA which inhibits the TGase activity.
**Biotin-cadaverine/CaCl₂ (R3)**
Lyophilized powder should be kept at -20°C. Lyophilised powder should be reconstituted with 6.5 mL of distilled water just before use. Unused solution should not be stored more than 4 hours at +4°C.

**Diluent buffer 10X (R5)**
The diluent buffer 10X should be kept at +4°C. Diluent buffer 10X should be diluted at 1/10 with distilled water just before use.

**Enzyme tracer (R4)**
Enzyme tracer should be kept at -20°C. Enzyme tracer should be diluted at 1/2000 with 1X diluent buffer just before use. Unused solution should be eliminated.

**HRP substrate (R6)/Chromogen (R7)**
The reagents should be kept at +4°C. 1 drop of chromogen should be added to 2 mL of HRP substrate just before use.

**Blocking reagent (R8)**
Blocking reagent should be kept at +4°C and is ready to use.

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**Assay procedure**

**Preliminary operations**

1. Identify a sufficient number of wells to run in duplicate:
   - the samples,
   - the positive control (TGase + distilled water)
   - the negative control (TGase + R2 reagent)

   Controls and samples should all be subjected to exactly the same assay procedure. Keep one blank well for HRP substrate/chromogen solution only.

2. Dispense 150 µL per well of 1X diluent buffer and incubate the plate for at least 30 minutes at 37°C.

3. Prepare the samples: samples from cells or tissues should be centrifuged before use.

**Perform the TG-Covtest as follows**

1. Remove the 1X diluent buffer from the plate.

2. Per well, dispense the samples* (50 µL), dispense the positive control (40µL TGase + 10µL distilled water) and dispense the negative control (40µL TGase + 10µL of R2 reagent).

3. Dispense 50 µL per well of reconstituted biotin-cadaverine/CaCl₂.

4. Incubate for 15 minutes at 37°C.

5. Prepare the enzyme tracer solution at 1/2000.

6. Wash the wells three times with Phosphate Buffered Saline containing 0.1% Tween 20 or Tris Buffered Saline containing 0.1% Tween 20.

7. Dispense 100 µL per well of 1/2000 diluted enzyme tracer solution.

8. Incubate for 15 minutes at 37°C.

9. Prepare the HRP substrate/chromogen solution.

10. Wash the wells three times with Phosphate Buffered Saline containing 0.1% Tween 20 or Tris Buffered Saline containing 0.1% Tween 20.

11. Dispense 100µL per well of HRP substrate/chromogen solution.

12. Incubate for 2 to 10 minutes at room temperature.

13. Dispense 50µL per well of blocking reagent.

14. Measure the optical density of each well at 450nm (OD450nm).

* Please note that it could be better to treat the samples as controls:
  - active TGase sample : (40µL sample + 10 µL distilled water) per well,
  - inactive TGase sample : (40µL sample + 10 µL R2 reagent) per well.

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**Warning**

This is a laboratory reagent. It is not to be administered to human or animals nor be used as a drug.
**Calculation of results**

Several experiments using purified guinea pig TGase (Activity: 2 units per mg protein) have shown that 1.25 mU/mL (final concentration) correspond to an absorbance value of $1.05 \pm 0.05$ OD at 450 nm. 1 unit will catalyze the formation of 1µmole of hydroxamate at pH 6.0 at 37°C, using L-glutamic acid γ-monohydroxamate as the standard (Folk and Cole, 1966). If other TGase standards are used in this assay the reference value may be different. End user should establish their own set of reference values.

**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 (R6), chromogen (R7) and blocking reagent (R8).
- R6, R7 and R8 reagents are irritating to eyes and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

**Example**

Comparaison of serial dilutions of commercial TGase and CovalAb's purified TGase

![Graph showing comparison of serial dilutions of commercial TGase and CovalAb's purified TGase.]

**Comparison of colorimetric and radiometric FXIII assays**

$y = -0.83314 + 32.718x \quad R^2 = 0.96036$

**References**