# Transglutaminase Activity Assay Kit (Colorimetric)

**Product name**: Transglutaminase Activity Assay Kit (Colorimetric)

**Detection method**: Colorimetric

**Sample type**: Tissue, Adherent cells, Suspension cells

**Assay type**: Enzyme activity

**Sensitivity**: 10 µU

**Assay time**: 2h 30m

**Product overview**

Transglutaminase Activity Assay Kit (ab204700) utilizes the deamidation reaction of the transglutaminase enzyme with a donor and acceptor substrate resulting in the formation of a hydroxamate product. The hydroxamate product reacts with the Stop Solution forming a purple complex that can be measured colorimetrically at 525 nm. The limit of quantification of this assay is ~10 µU in cell/tissue lysates.

Transglutaminase assay protocol summary:
- add samples and standards to wells
- add reaction mix and incubate for 2 hrs
- add stop solution and centrifuge plate at 1800 g for 15 min
- transfer supernatant to fresh plate
- analyze with microplate reader

**Notes**

Transglutaminases (EC 2.3.2.13) are calcium dependent enzymes that catalyze the post-translational modification of proteins by formation of isopeptide bonds. This occurs either through protein cross-linking via formation of γ-glutamyl-ε-lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. The transglutaminase enzyme family comprises the intracellular forms (TG1, TG3 and TG5) expressed mostly in the epithelial tissue; TG2 which is both intracellular and extracellular and expressed in various tissue types; TG4 which is expressed in the prostate gland; factor XIII which is expressed in blood; TG6 and TG7, whose tissue distribution is unknown and band 4.2 (lacking enzymatic activity) which is present on erythrocyte membranes. Transglutaminases also exhibit GTPase, phosphodiesterase and protein kinase activity. Transglutaminases are associated with certain neurological and autoimmune disorders and also cancer.

**Platform**

Microplate reader

**Properties**
Storage instructions
Store at -20°C. Please refer to protocols.

<table>
<thead>
<tr>
<th>Components</th>
<th>100 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptor Substrate</td>
<td>2 vials</td>
</tr>
<tr>
<td>Donor Substrate</td>
<td>1 vial</td>
</tr>
<tr>
<td>DTT</td>
<td>1 x 125µl</td>
</tr>
<tr>
<td>Homogenization Buffer (10x)</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Hydroxamate Standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>1 unit</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 vial</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 5ml</td>
</tr>
<tr>
<td>TG Assay Buffer</td>
<td>1 x 12ml</td>
</tr>
</tbody>
</table>

Relevance
Transglutaminases (EC 2.3.2.13) are calcium dependent enzymes that catalyze the post-translational modification of proteins by formation of isopeptide bonds. This occurs either through protein cross-linking via formation of ε-glutamyl-ε-lysine bonds or through incorporation of primary amines at selected peptide-bound glutamine residues. The transglutaminase enzyme family comprises the intracellular forms (TG1, TG3 and TG5) expressed mostly in the epithelial tissue; TG2 which is both intracellular and extracellular and expressed in various tissue types; TG4 which is expressed in the prostate gland; factor XIII which is expressed in blood; TG6 and TG7, whose tissue distribution is unknown and band 4.2 (lacking enzymatic activity) which is present on erythrocyte membranes. Transglutaminases also exhibit GTPase, phosphodiesterase and protein kinase activity. Transglutaminases are associated with certain neurological and autoimmune disorders and also cancer.

Images
Typical Hydroxamate Standard calibration curve.

Transglutaminase Activity Assay Kit (ab204700)
Transglutaminase activity in HepG2 cells (human hepatoblastoma cell line) and rat liver lysate: HepG2 cells were stimulated with vehicle (DMSO), IL6 (1 µM), Dexamethasone (DXM -1 µM), or with IL6 (1µM) and DXM (1 µM). Approximately 250 µg protein was used for determining transglutaminase activity in cells and tissue lysate. Activity is expressed as nmols of product formed in 2h and is normalized to the protein amount.

Note: HepG2 cells have similar intrinsic Transglutaminase activity in the presence or absence of vehicle control (DMSO).

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