ab204700

Transglutaminase Activity Assay Kit (Colorimetric)

Instructions for Use

For rapid, sensitive and accurate measuring of Transglutaminase activity.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 6c, Last Updated 7 February 2024

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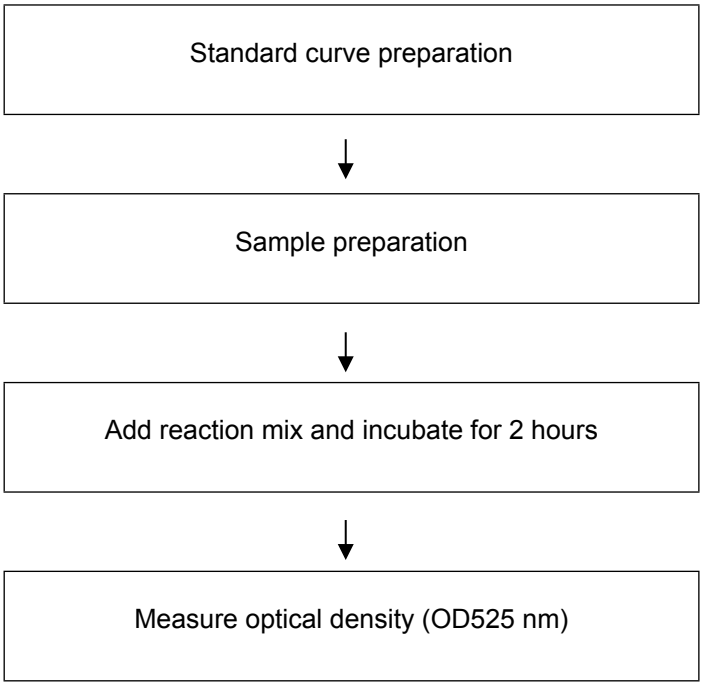
1. BACKGROUND

Transglutaminase Activity Assay Kit (ab204700) uses 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 TG Stop Solution/Stop Solution forming a purple complex that can be measured colorimetrically at OD = 525 nm. The limit of quantification of this assay is ~10 µU or 80 ng of recombinant hTG2 enzyme.

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.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
TG Assay Buffer	12 mL	-20°C	-20°C
10X Homogenization Buffer/Homogenization Buffer (10x)	10 mL	-20°C	-20°C
DTT I/DTT	150 µL	-20°C	-20°C
TG Donor Substrate/Donor Substrate	1 Vial	-20°C	-80°C
Acceptor Substrate	2 Vials	-20°C	-80°C
Hydroxamate Standard	1 Vial	-20°C	-20°C
TG Stop Solution/Stop Solution	5 mL	-20°C	-20° / +4C
TG Positive Control/Positive Control	1 Vial	-20°C	-80°C
Microplate Sealing Film/Plate Sealer	1 each	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 525 nm
- 96 well plate: clear plates
- Heat block or water bath
- Dounce homogenizer or pestle (if using tissue)
- Glycerol – prepare a 20% glycerol solution in ddH_2O
- Protease Inhibitor Cocktail (ab201111 or equivalent)

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **TG Assay Buffer:**

Ready to use as supplied. Equilibrate to 37°C before use. Store at -20°C.

9.2 **10X Homogenization Buffer/Homogenization Buffer (10X):**

Make 1X Homogenization buffer by diluting 1:10 in ddH_2O.Store at -20°C. Keep on ice while in use.

9.3 **DTT I/1 M DTT:**

Ready to use as supplied. Aliquot DTT I/DTT so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.4 **TG Donor Substrate/Donor Substrate:**

Reconstitute the TG Donor Substrate/Donor Substrate in 1.1 mL of ddH_2O. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Once the probe is thawed, use within two months.

9.5 **Acceptor Substrate:**

Reconstitute each vial of the Acceptor Substrate in 550 µL of ddH_2O as needed. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Once the probe is thawed, use with two months.

9.6 **Hydroxamate Standard:**

Reconstitute the Hydroxamate Standard in 330 µL of ddH_2O to generate a 10 mM standard stock solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use.

9.7 **TG Stop Solution/Stop Solution:**

Ready to use as supplied. Store at -4°C or -20°C. Keep on ice while in use.

9.8 **TG Positive Control/Positive Control:**

Reconstitute the TG Positive Control/Positive Control in 30 µL of 20% glycerol in ddH_2O. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -80°C protected from light. Thaw and mix gently before use.

9.9 **Microplate Sealing Film/Plate Sealer:**

Ready to use as supplied. Equilibrate to room temperature before use.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and cannot be stored for future use.

10.1 Dilute TG Assay Buffer 1:1 with ddH_2O.

10.2 Using 10 mM Hydroxamate standard, prepare standard curve dilution as described in the table in a separate microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Diluted TG Assay Buffer (µL)	Final volume standard in well (µL)	End Conc. Hydroxamate (nmol in well)
1	0	150	50	0
2	6	144	50	20
3	12	138	50	40
4	18	132	50	60
5	24	126	50	80
6	30	120	50	100

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Add DTT I/DTT to 1X Homogenization Buffer at a final concentration of 0.2mM.

11.1.2 Harvest the amount of cells necessary for each assay (initial recommendation = 2-5 x 10^6 cells).

11.1.3 Wash cells with cold PBS.

11.1.4 Add 150 – 300 µL cold Homogenization Buffer (with DTT) containing protease inhibitor cocktail.

11.1.5 Disrupt cells by five cycles of freezing and thawing.

11.1.6 Transfer cells to cold microcentrifuge tube.

11.1.7 Centrifuge sample for 20 minutes at 4°C at 16,000 x g using a cold microcentrifuge.

11.1.8 Collect supernatant and transfer to a clean pre-chilled tube and keep on ice.

11.2 Tissue samples:

11.2.1 Add DTT I/DTT to 1X Homogenization Buffer at a final concentration of 0.2mM.

11.2.2 Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg).

11.2.3 Rinse tissue with PBS and transfer tissue to pre-chilled homogenizer.

11.2.4 Add 500 µL cold Homogenization Buffer (with DTT) containing protease inhibitor cocktail.

11.2.5 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.6 Transfer tissue to cold microcentrifuge tube.

11.2.7 Centrifuge sample for 20 minutes at 4°C at 16,000 x g using a cold microcentrifuge.

11.2.8 Collect supernatant and transfer to a clean pre-chilled tube and keep on ice.

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

To check the Transglutaminase activity, protein concentration of lysates should be at least 5 mg/mL.

We recommend using the lysates immediately to assay the Transglutaminase Activity.

12. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

12.1 **Set up Reaction wells:**

- Sample wells = 25 – 50 µL samples (adjust volume to 50 µL/well with ddH_2O).
- Background control sample wells= 50 µL ddH_2O.
- Positive control = 2 µL TG Positive Control/Positive control + 48 µL ddH_2O. ***NOTE:*** *add positive control to plate just before adding Transglutaminase Reaction Mix.*

12.2 **Reaction Mix:**

Prepare 50 µL of Transglutamate Reaction Mix for each reaction:

Component	Reaction Mix (µL)
TG Assay Buffer	**25**
TG Donor Substrate/Donor Substrate	**10**
Acceptor Substrate	**10**
DTT I/1M DTT	**1**
ddH_2O	**4**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X µL component x (Number reactions +1).

12.3 Add 50 µL of Reaction Mix into each sample, positive control and background control sample wells.

12.4 Mix and incubate at 37°C for 2 hours protected from light.

12.5 Set up Standard wells in a separate 96-well plate towards the end of the 37ºC 2 hours incubation of the Sample plate = add 50 µL standard dilutions.

12.6 Add 50 µL TG Stop Solution/Stop solution to all Standards and sample wells.

12.7 Seal the sample plate, and centrifuge at 1800 x g for 15 minutes to pellet the precipitate formed. ***NOTE:*** *Standard will not precipitate after adding stop solution.*

12.8 Carefully transfer 100 µL of supernatant from all sample and background control wells into desired wells in the 96-well clear plate containing the standards.

12.9 Measure output at OD = 525 nm on a colorimetric microplate reader.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 If the sample background control is significant, then subtract the sample background control from sample reading.

13.3 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4 Plot the corrected absorbance values for each standard as a function of the final concentration of hydroxamate.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Activity of Transglutamate (nmol/T/mg) in the test samples is calculated as:

$$Transglutaminase\ activity = \frac{((B * 1.5)/T)}{mg}$$

Where:

B = Amount of hydroxamate from the Standard Curve (nmol).

1.5 = nmoles of hydroxamate product generated in 150 µL reaction volume.

T = Incubation time (minutes).

mg = Amount of protein/reaction in mg

Unit Definition:

1 Unit Transglutaminase activity = amount of enzyme which generates 1.0 µmol of hydroxamate per minute under the assay conditions.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

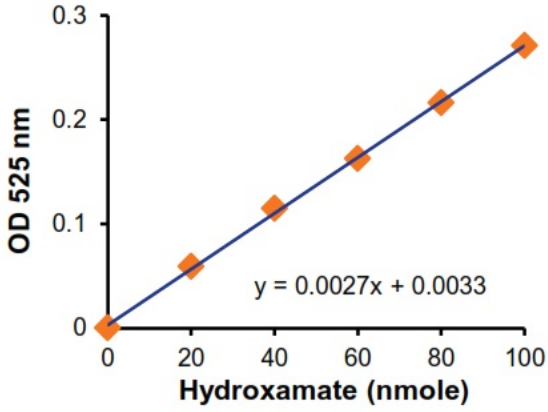


Figure 1. Typical Hydroxamate Standard calibration curve.

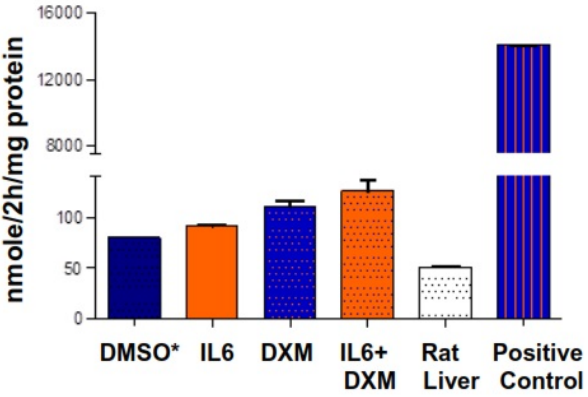


Figure 2. Transglutaminase activity in HepG2 cells (human hepatoblastoma cell line) and rat liver lysate: HepG2 cells were stimulated with vehicle (DMSO), IL6 (1 µM), Dexamethasone (DXM -1 µM), or with IL6 (1µM) and DXM (1 µM). Approximately 250 µg protein was used for determining transglutaminase activity in cells and tissue lysate. Activity is expressed as nmoles 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).*

15. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, donor substrate, acceptor substrate, and positive control (aliquot if necessary); get equipment ready.
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up 2 plates: one for standard (50 µL) and one for samples (50 µL), positive control (50 µL) and background wells (50 µL).
- Prepare Transglutaminase Reaction Mix (Number samples + positive control + background control + 1).

Component	Colorimetric Reaction Mix (µL)
TG Assay Buffer	**25**
TG Donor Substrate/Donor Substrate	**10**
Acceptor Substrate	**10**
DTT I/1M DTT	**1**
ddH2O	**4**

- Add 50 µL of Transglutaminase Reaction Mix to the samples, positive control and background wells.
- Incubate plate at 37°C 2 hours protected from light.
- Add 50 µL of TG Stop Solution/Stop Solution to the samples, positive control and background wells and to all standards.
- Centrifuge at 1800 x g for 15 minutes to pellet the precipitate formed.
- Transfer 100 µl of supernatant from all sample, positive control and background control wells into the 2nd 96-well clear plate containing the standards.

- Measure plate at OD = 525 nm.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. FAQ

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)