

APB742Mi01 100µg Active UDP Glucose Ceramide Glucosyltransferase (UGCG) Organism Species: Homo sapiens (Human) Mus musculus (Mouse) Rattus norvegicus (Rat) Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Lys39~Leu171

Tags: N-terminal His-tag

Purity: >95%

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.7

Predicted Molecular Mass: 16.1kDa

Accurate Molecular Mass: 16kDa as determined by SDS-PAGE reducing conditions. Note: 98% cross-reactivity of UGCG was observed among human, mouse and rat.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

KK ATDKQPYSKL PGVSLLKPLK GVDPNLINNL ETFFELDYPK YEVLLCVQDH DDPAIDVCKK LLGKYPNVDA RLFIGGKKVG INPKINNLMP GYEVAKYDLI WICDSGIRVI PDTLTDMVNQ MTEKVGLVHG L

[ACTIVITY]

UDP Glucose Ceramide Glucosyltransferase (UGCG) is an enzyme which catalyzes the first glycosylation step in glycosphingolipid biosynthesis. It belongs to the glycosyltransferase 2 family. UGCG is widely expressed and transcription is upregulated during keratinocyte differentiation. Besides, Large Multifunctional Peptidase 2 (LMP2) has been identified as an interactor of UGCG, thus a binding ELISA assay was conducted to detect the interaction of recombinant human UGCG and recombinant human LMP2. Briefly, UGCG were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to LMP2-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-UGCG pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were

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incubated 15-25 minutes at 37° C. Finally, add 50μ L stop solution to the wells and read at 450nm immediately. The binding activity of UGCG and LMP2 was shown in Figure 1, and this effect was in a dose dependent manner.

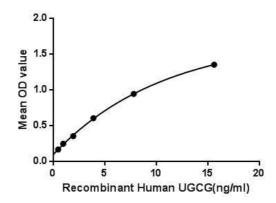
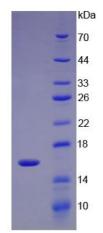
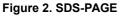


Figure 1. The binding activity of UGCG with LMP2.

[IDENTIFICATION]





Sample: Active recombinant UGCG, Human

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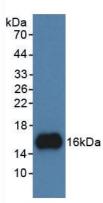


Figure 3. Western Blot

Sample: Recombinant UGCG, Human;

Antibody: Rabbit Anti-Human UGCG Ab (PAB742Mi01)