GENLISA® Total Carbohydrate Assay

REF : KBCA2246

Ver 1.1

RUO

Fluorescent based Biochemical Assay for the Quantitative Determination of Total Carbohydrate in animal tissues and cell culture samples

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

For Research Use Only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of Krishgen Biosystems Private Limited is strictly prohibited.

REF KBCA2246 Σ 96 tests

Krishgen Biosystems Private Limited
For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales1@krishgen.com | http://www.krishgen.biz / www.krishgenbio.com

Background:

Carbohydrate is one of the important components of various bodies, and is also the main raw material and storage material of metabolism. Total carbohydrate mainly refers to reducing glucose, fructose, pentose, lactose, maltose, sucrose that can be hydrolyzed to reducing monosaccharides under determined conditions, and possibly partially hydrolyzed starch.

Intended Use:

The GENLISA® Total Carbohydrate Assay can measure total carbohydrate content in serum, plasma, animal and plant tissue samples.

Principle:

GENLISA® Total Carbohydrate Assay is an assay kit where the total carbohydrate is hydrolyzed to reducing sugar, which is reduced to amino compounds after co-heating with 3, 5- dinitrosalicylic acid under alkaline conditions. This reaction produces a reddish-brown compound, the intensity of which is directly proportional to the concentration of reducing sugars, and thereby to the total carbohydrate content in the sample.

Materials Provided:

1. Blank Microplate - 96 wells
2. Standard (Powder) – 1 vial
3. Extraction Solution A - 55 ml
4. Extraction Solution B - 55 ml
5. Chromogenic Agent - 4 ml

Materials Provided by the End-user:

1. Fluorescence Microtiter Plate Reader with Ex/Em 340nm/440nm.
2. Incubator.
3. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
4. PBS (0.01M, pH7.4).
5. Deionized (DI) water.
6. Wash bottle or automated microplate washer.
7. Graph paper or software for data analysis.
8. Timer.
9. Absorbent Paper.

Handling/Storage:

1. All reagents should be stored as indicated on the component label at -20ºC, away from light.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. All the components in the kit are light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only

Reagent Preparation

1. Bring all the reagents to room temperature 25ºC before use.

2. Before testing, incubate the chromogenic agent in 75ºC water bath, and then cool it to 25ºC under the running water.

3. The preparation of 1 mg/ml standard solution:

   Before testing, prepare sufficient 1 mg/ml standard solution. For example, prepare 1000 ul of 1 mg/ml standard solution i.e. 100 ul of 10 mg/ml and 900 ul of double distilled water.

4. **Standards Preparation:**
   Reconstitute one vial of standard with 1 ml of double distilled water, mix well to dissolve. Store at 2-8°C for 1 month.
   Dilute the above reconstituted standard (1 mg/ml) with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0 mg/ml.

Item	1	2	3	4	5	6	7	8
Concentration (mg/ml)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
1 mg/ml standard (ul)	0	20	40	60	80	120	160	200
Double distilled water (ul)	200	180	160	140	120	80	40	0

Note: Always prepare a fresh set of standards. Discard working standard dilutions immediately after use.

Sample Preparation

Serum or plasma samples:
1. Take 0.1 ml of serum (plasma) to 2 ml tube. Add 0.1 ml of extraction solution A and 0.15 ml of double distilled water into the tube.
2. Mix well, and incubate the tubes in 95ºC water bath for 30 min. Cool the tubes to 25ºC under running water.
3. Add 0.1 ml of extraction solution B, mix well.
4. Centrifuge at 8000×g for 10 min at 25ºC to remove insoluble material. Collect supernatant and keep it on ice for detection.

Tissue Sample:
1. Harvest the amount of tissue needed for each assay (initial recommendation 50 mg).
2. Wash tissue in cold PBS (0.01 M, pH 7.4).
3. Take 50 mg of tissue to 2 ml tube. Add 0.5 ml of extraction solution A and 0.75 ml of double distilled water into the tube with a dounce homogenizer at 4°C.
4. Incubate in 95°C water bath for 30 min.
5. Add 0.5 ml of extraction solution B, mix well.
6. Centrifuge at 8000xg for 10 min at 25°C to remove insoluble material. Collect the supernatant and place on ice for detection.

Sample Dilution:

The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
10% Wheat Tissue Homogenate	30-60
10% Paddy Tissue Homogenate	30-60
10% Corn Tissue Homogenate	30-60
10% Pumpkin Tissue Homogenate	30-50
10% Mouse Liver Tissue Homogenate	3-10
Human Serum	1

Note:
The diluent is extraction solution A. For the dilution of other sample types, please do pretest to confirm the dilution factor.

Assay Procedure

1. Add 30 ul of prepared standard to the respective tubes.
2. Add 30 ul of sample to the respective tubes.
3. Add 30 ul of chromogenic agent to each tube and mix well.
4. Incubate the tubes in 95°C water bath for 30 min, and cool it to 25°C under running water.
5. Add 180 ul of double distilled water to each tube.
6. Mix thoroughly and take 200 ul of reaction solution to the microplate. Measure the OD value of each well at 540 nm with microplate reader.

Calculation of Results:

The standard curve:

1. Average the duplicate reading for each standard.
2. Subtract the mean OD value of the blank (Standard #1) from all standard readings. This is the absoluted OD value.
3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or EXCEL).

The sample:

1. Serum and plasma samples:

$$\text{total carbohydrate content (mg/ml)} = \frac{\Delta A - b}{a} \times V_2 \div V_3 \times f$$

2. Tissue Sample:

$$\text{total carbohydrate content (mg/g wet weight)} = \frac{\Delta A - b}{a} \times V_1 \div m \times f$$

Note:

ΔA: $\Delta A = A_{sample} - A_{blank}$.

V_1: Total volume of tissue sample after treatment, 1.75 ml

m: The wet weight of sample, 0.05 g.

V_2: Total volume of serum (plasma) or liquid sample after treatment, 0.45 ml.

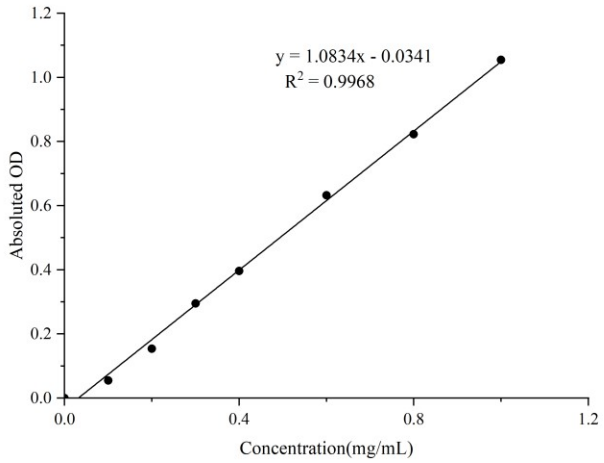
V_3: The volume of serum (plasma) or liquid sample, 0.1 ml.

f: Dilution factor of sample before test.

Typical Data

Concentration (mg/ml)	0	0.1	0.2	0.3	0.4	0.6	0.8	1.0
OD Value	0.055	0.109	0.215	0.352	0.458	0.675	0.882	1.104
	0.055	0.111	0.203	0.349	0.444	0.700	0.874	1.115
Average OD	0.055	0.110	0.209	0.351	0.451	0.688	0.878	1.110
Absoluted OD	0.000	0.055	0.154	0.296	0.396	0.633	0.823	1.055

Typical Graph



Quality Control:
It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:
This kit has been validated. Please view the details herein below.

Sensitivity
The analytical sensitivity of the assay is 0.036 mg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Intra-Assay Precision
Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/ml)	0.5	0.7	0.9
%CV	1.0	1.9	2.9

Inter-Assay Precision
Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mg/ml)	0.25	0.50	0.75
%CV	2.1	4.5	5.3

Recovery
Three samples of high concentration, middle concentration and low concentration were tested 6 times to get the mean recovery rate of 104.7%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mg/ml)	0.5	0.7	0.9
Observed Conc. (mg/ml)	0.55	0.74	0.93
Recovery Rate (%)	105.0	106.0	103.0

LIMITED WARRANTY

Krishgen Biosystems Private Limited does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the Products; against defects in products or components not manufactured by Krishgen Biosystems Private Limited, or against damages resulting from such non-Krishgen Biosystems Private Limited made products or components. Krishgen Biosystems Private Limited passes on to customer the warranty it received (if any) from the maker thereof of such non Krishgen made products or components. This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems Private Limited.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems Private Limited shall be to repair or replace the defective Products in the manner and for the period provided above. Krishgen Biosystems Private Limited shall not have any other obligation with respect to the Products or any part thereof, whether based on contract, tort, and strict liability or otherwise. Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems Private Limited be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems Private Limited with respect to the Products. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems Private Limited. 2025

THANK YOU FOR USING A KRISHGEN PRODUCT!

KRISHGEN BIOSYSTEMS PRIVATE LIMITED | OUR REAGENTS | YOUR RESEARCH |