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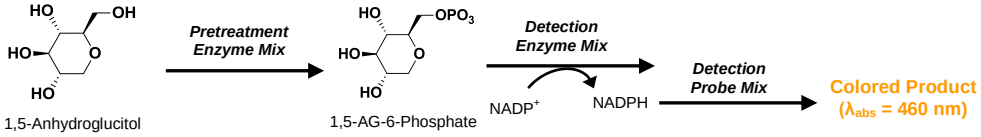
1,5-Anhydroglucitol Assay Kit (Colorimetric)

11/19

(Catalog # K2029-100; 100 Assays; Store at -20°C)

I. Introduction:

1,5-Anhydroglucitol (1,5-AG) is a metabolically inactive deoxy analogue of glucose that is present in most grains and meats. It is well absorbed in the intestine from dietary sources and is readily reabsorbed by the Na^+-dependent glucose symporter (SGLT4) in renal proximal tubules. A small amount of 1,5-AG (proportional to dietary intake) is released in the urine, resulting in a constant steady-state concentration in blood and most tissues (variable depending on diet). However, in hyperglycemia (plasma glucose above 180 mg/dl), glucose saturates SGLT4, thereby preventing 1,5-AG reuptake and dramatically increasing the urinary excretion of 1,5-AG. Measurement of plasma or saliva 1,5-AG levels can identify recent (within the last 10-14 days) hyperglycemic episodes (even if glucose levels appear normal at the time of measurement) and reflects the mean blood glucose level over the previous 1-2 weeks in diabetic patients. **BioVision's 1,5-Anhydroglucitol Assay Kit** is a high-throughput adaptable microplate-based assay that enables rapid quantification of 1,5-AG levels in biological fluids. The assay consists of two steps: in the first step, the sample is pretreated to remove endogenous glucose that may interfere with the 1,5-AG detection and 1,5-AG is converted to 1,5-AG-6-phosphate. In the second step, 1,5-AG-6-phosphate is enzymatically metabolized, generating NADPH, which subsequently reacts with a colorless probe to form a strongly colored product measured by absorbance at OD = 460 nm. The absorbance is proportional to the concentration of 1,5-AG present in the samples. The assay is not affected by galactose, fructose or other physiologically common sugars and has a limit of detection of 0.11 nmoles of 1,5-AG per well, with a reliable linear range from 0.2 – 5 nmole per well (corresponding to a 20-500 µM concentration in a 10 µl sample volume).



II. Applications:

- Estimation of 1,5-anhydroglucitol concentration in biological fluids (serum/plasma, saliva)

III. Sample Type:

- Human or animal plasma/serum or saliva

IV. Kit Contents:

Components	K2029-100	Cap Code	Part Number
Sample Pretreatment Buffer	10 ml	NM	K2029-100-1
Detection Buffer	10 ml	Blue/NM	K2029-100-2
Pretreatment Enzyme Mix	1 vial	White	K2029-100-3
Pretreatment Cofactor Mix	1 vial	Orange	K2029-100-4
Detection Enzyme Mix	1 vial	Green	K2029-100-5
Detection Probe	1 vial	Red	K2029-100-6
1,5-AG Standard	1 vial	Yellow	K2029-100-7
Half-Area 96-Well Plate	1 plate	N/A	K2029-100-8

V. User Supplied Reagents and Equipment:

- Multiwell microplate spectrophotometer (capable of reading absorbance at 460 nm)
- 0.2 µm Syringe Filter (*e.g.* BioVision Cat # M4274 or equivalent) for clarification of turbid or lipemic samples

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C and protect from light. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay procedure.

- **Sample Pretreatment Buffer** and **Detection Buffer:** Warm to room temperature (RT) before use.
- **Pretreatment Enzyme Mix, Pretreatment Cofactor Mix** and **Detection Enzyme Mix:** Reconstitute each vial with 220 µl of ddH_2O. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles.
- **Detection Probe:** Reconstitute the vial with 220 µl of ddH_2O. Divide into aliquots and store at -20°C, **protected from light.** Avoid repeated freeze/thaw cycles.
- **1,5-AG Standard:** Reconstitute with 220 µl of ddH_2O for a 5 mM stock 1,5-AG Standard solution. Store at -20°C, stable for 4 freeze/thaw cycles.

VII. 1,5-Anhydroglucitol Assay Protocol:

1. Sample Preparation:

a. Collect serum/plasma or saliva samples by standard methods (see notes below regarding compatible blood collection tubes and anticoagulants below) and filter using a 0.2 µm syringe filter in order to eliminate lipid globules and other debris.

b. Add 5-20 µl of undiluted sample to the desired well(s) in the provided half-area 96-well plate. Adjust the volume of all sample wells to 30 µl/well with Sample Pretreatment Buffer.

Notes:

- We recommend using either "off-the-clot" serum (collected in tubes that are free of anticoagulants) or plasma collected with lithium/sodium heparin.

- To ensure accurate determination of 1,5-AG in test sample types that are expected to have a low concentration of 1,5-AG (such as saliva samples), we recommend running two parallel Sample wells and spiking one with a known amount of 1,5-AG Standard (1 nmole). For the Spiked Sample, add the same volume of sample as in the test well and add 2 µl of 500 µM 1,5-AG Standard (see section VII.2 below). Adjust volume to 30 µl with Sample Pretreatment Buffer.

2. **Standard Curve Preparation:** Prepare a working 500 µM 1,5-AG Standard solution by adding 20 µl of the stock 5 mM 1,5-AG Standard to 180 µl of ddH_2O. Add 0, 2, 4, 6, 8, and 10 µl of the working 500 µM 1,5-AG Standard solution into a series of wells, generating 0, 1, 2, 3, 4 and 5 nmol of 1,5-AG Standard/well. Adjust the volume of all 1,5-AG Standard wells to 30 µl/well with Sample Pretreatment Buffer.

3. **Reaction Mix Preparation:**

a. Prepare Pretreatment Reaction Mix for Sample and Standard Curve wells according to the table below. Make sufficient amount of the Pretreatment Reaction Mix to add 20 µl to all assay wells.

	Pretreatment Reaction Mix
Sample Pretreatment Buffer	16 µl
Pretreatment Enzyme Mix	2 µl
Pretreatment Cofactor Mix	2 µl

b. Add 20 µl of Pretreatment Reaction Mix to all Sample, Standard Curve and Spiked Sample wells, bringing the volume to 50 µl/well.

c. Incubate the plate for 90 min at 37°C, protected from light.

d. Prepare Detection Reaction Mix according to the table below. Make a sufficient amount of the Detection Reaction Mix to add 50 µl to all assay wells.

	Detection Reaction Mix
Detection Buffer	46 µl
Detection Enzyme Mix	2 µl
Detection Probe	2 µl

e. Add 50 µl of Detection Reaction Mix to all wells, bringing the final volume to 100 µl/well.

f. Incubate the plate for 60 min at 37°C, protected from light.

4. **Measurement:** Following 60 min incubation, measure the absorbance of all Sample, Spiked Sample and Standard wells at 460 nm in endpoint mode.

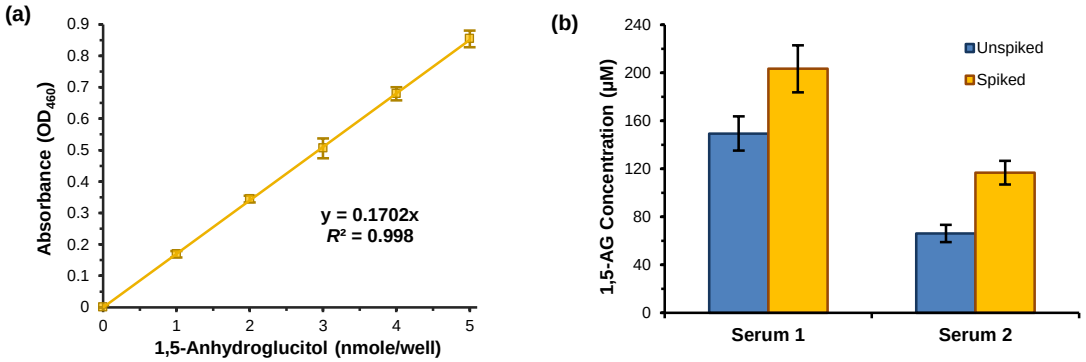
5. **Calculations:** For the 1,5-AG Standard Curve, subtract the Reagent Blank (0 nmoles/well) absorbance reading from each of the Standard readings. Plot the Reagent Blank-subtracted absorbance values and calculate the slope of the 1,5-AG Standard Curve. For Samples, calculate the corrected Sample absorbance (A_c) by subtracting the Reagent Blank absorbance from the Sample absorbance: $\boldsymbol{A_c = (OD_{460})_{Sample} - (OD_{460})_{Reagent\ Blank}}$. Apply the A_c values to the Standard Curve to get *B* nmoles of 1,5-AG in the Sample well(s).

$$\textbf{Sample 1,5-Anhydroglucitol concentration} = \frac{\boldsymbol{B}}{\boldsymbol{V}} \times \boldsymbol{D} = \textbf{nmol/µl = mM}$$

Where: ***B*** is the amount of 1,5-AG, calculated from the Standard Curve (in nmole)
V is the volume of Sample added to the well (in µl)
D is the Sample dilution factor (if applicable, *D* = 1 for undiluted Samples)

Note: For Spiked Samples, subtract the Reagent Blank absorbance from the Sample reading and the Spiked Sample reading. Calculate ***B*** using the corrected Sample reading (A_c) and the corrected Spiked Sample reading ($A_{c+spike}$), according to the formula:

$$\textbf{1,5-AG in Sample (with internal spike) } (\boldsymbol{B}) = \left(\frac{\boldsymbol{A_c}}{(\boldsymbol{A_{c+spike}}) - \boldsymbol{A_c}}\right) = \textbf{nmol/µl = mM}$$



Figures: (a) 1,5-Anhydroglucitol (1,5-AG) Standard Curve. 1,5-AG concentration is directly proportional to the absorbance measured at 460 nm. **(b)** Estimation of 1,5-AG in two different single-donor "off-the-clot" human serum samples (10 µl), each spiked with 50 µM 1,5-AG. Mean 1,5-AG concentrations detected in the samples were 149.4 ± 14.2 µM and 66.1 ± 7.2 µM, respectively (mean spike recovery rates for the two samples were 107.9% and 101.6%, respectively). Data are mean ± SD of 4-5 replicates, assayed according to the kit protocol.

VIII. Related Products:

Glucose Assay Kit (K606) | Glucose-6-Phosphate Assay Kit (K657) | Fructosamine Assay Kit (K450)
Advanced Glycation End Products Assay Kit (K929) | Fructose Assay Kit (K619) | PicoProbe™ Glucose Fluorometric Assay Kit (K688)

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