ab284531 – 1,5-Anhydroglucitol Activity Assay Kit (Colorimetric)

For the quantitative measurement of 1,5-Anhydroglucitol in biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284531

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.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 1 year.

Materials Supplied

Item	Quantity	Storage Condition
1,5-AG Standard	1 vial	-20°C
Detection Buffer	10 mL	-20°C
Detection Enzyme Mix	1 vial	-20°C
NADP Detection Probe/Detection Probe	1 vial	-20°C
96-Well Half Area Plate/Half-Area 96-Well Plate	1 unit	-20°C
Pretreatment Cofactor Mix	1 vial	-20°C
Pretreatment Enzyme Mix	1 vial	-20°C
Sample Pretreatment Buffer	10 mL	-20°C

Materials Required, Not Supplied

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

- Multi-well microplate spectrophotometer
- 0.2 µm Syringe Filter for clarification of turbid or lipemic samples

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

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.

NADP Detection Probe/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.

Assay Protocol

Sample Preparation:

1. 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.
2. Add 5-20 µl of undiluted sample to the desired well(s) in the provided 96-Well Half Area Plate/half-area 96-well plate. Adjust the volume of all sample wells to 30 µl/well with Sample Pretreatment Buffer.

Δ Notes:

a. *We recommend using either "off-the-clot" serum (collected in tubes that are free of anticoagulants) or plasma collected with lithium/sodium heparin.*
b. *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. Adjust volume to 30 µl with Sample Pretreatment Buffer.*

Standard Curve Preparation:

1. 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 dH_2O.
2. 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.
3. Adjust the volume of all 1,5-AG Standard wells to 30 µl/well with Sample Pretreatment Buffer.

Reaction Mix:

1. 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:

Item	Reaction Mix
Sample Pretreatment Buffer	16 µL
Pretreatment Enzyme Mix	2 µL
Pretreatment Cofactor Mix	2 µL

2. Add 20 µl of Pretreatment Reaction Mix to all Sample, Standard Curve and Spiked Sample wells, bringing the volume to 50 µl/well.
3. Incubate the plate for 90 min at 37°C, protected from light.
4. 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.

Item	Detection Reaction Mix
Detection Buffer	46 µL
Detection Enzyme Mix	2 µL
NADP Detection Probe/Detection Probe	2 µL

5. Add 50 µl of Detection Reaction Mix to all wells, bringing the final volume to 100 µl/well.
6. Incubate the plate for 60 min at 37°C, protected from light.

Measurement

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

Calculation:

1. **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.**
2. **For Samples, calculate the corrected Sample absorbance (A_C) by subtracting the Reagent Blank absorbance from the Sample absorbance: $A_C = (OD_{460})_{Sample} - (OD_{460})_{Reagent\ Blank}$. Apply the A_C values to the Standard Curve to get B nmoles of 1,5-AG in the Sample well(s).**

Sample 1,5-Anhydroglucitol concentration = $\frac{B}{V}\ \mathbf{X\ D}\ = \mathbf{nmol/\mu L}\ = \mathbf{mM}$

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

Δ 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:

1,5-AG in Sample (with internal spike) (B) = $\left(\frac{\mathbf{Ac}}{(\mathbf{Ac+spike})-(\mathbf{Ac})}\right)\ = \mathbf{nmol/\mu L}\ = \mathbf{mM}$

Technical Support

For all technical or commercial enquiries please go to:

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