ab284547 – Glutaminase (GLS) Activity Assay Kit (Fluorometric)

For the measuring of glutaminase activity in biological samples. For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.**abcam.com**/ab284547

Introduction

Glutaminase (EC 3.5.1.2) is a mitochondrial enzyme that hydrolyzes glutamine producing glutamate and ammonia. GLS is one of the two key enzymes that are responsible for glutamine homeostasis, the other being glutamine synthetase. Since glutamine is a major metabolic substrate involved in gluconeogenesis, its homeostasis is tightly controlled. Glutaminase has tissue-specific roles in multiple organs, with the isoform GLS1 being expressed in kidneys and brain, and GLS2 being expressed in the liver. It is involved in maintenance of acid-base homeostasis by producing ammonia during renal acidosis in kidneys; and regulation of glutamate –which acts as a neurotransmitter- in the brain. On the other hand, GLS 2 regulates the generation of ammonia in liver by producing urea. Research shows GLS increased activity in individuals facing starvation, diabetes and high protein diets. This Glutaminase (GLS) Activity Assay Kit (ab284547) (previously called PicoProbe Glutaminase (GLS) Activity Assay Kit K455) is a simple plate-based fluorometric assay for measuring glutaminase activity in biological samples. GLS hydrolyzes glutamine forming glutamate and ammonia. Glutamate, in the presence of a developer and enzyme mix converts a non-fluorescent probe to a fluorescent product via an enzymatic reaction. The assay can detect as low as 2 µU of glutaminase in biological samples.

Applications

Measurement of Glutaminase activity in cell and tissue lysates using a 96-well plate format.

Sample Types

- Tissue lysate (e.g. Liver tissue)
- Recombinant enzyme
- Isolated Mitochondria
- Purified protein

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
GLS Assay Buffer	35 mL	-20°C
Converter Mix A	1 vial	-20°C
GLS Dilution Buffer	200 µL	-20°C
Developer Mix E	1 vial	-20°C
GLS Positive Control	10 µL	-20°C
GLS Substrate	2 vials	-20°C
Glutamate Standard	0.1 mL	-20°C
PicoProbe II	0.2 mL	-20°C

PLEASE NOTE: Converter Mix A was previously labelled as Converter Mix I and GLS Developer, and Developer Mix E as Development Enzyme Mix VI and GLS Enzyme Mix. The composition has not changed.

Materials Required, Not Supplied

- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- dry DMSO
- 10 kDa cutoff spin filters (Cat. No. ab93349)

Reagent Preparation

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

GLS Assay Buffer: Warm to room temperature before use.

GLS Dilution Buffer: Keep on ice when in use.

GLS Substrate: Reconstitute GLS Substrate, one vial at a time, in 220 µL water. Heat at 37°C in a water bath for 15-20 minutes to allow the substrate to dissolve complexly followed by vortexing. Aliquot and store at -20°C in the dark. Thaw at room temperature before use. If precipitates are observed, heat at 37°C in a water bath for 15 minutes and vortex.

Converter Mix A and Developer Mix E: Reconstitute each vial with 220 µL GLS assay buffer. Aliquot and store the remaining at -20°C in the dark. Thaw Converter Mix A and Developer Mix E on ice before use.

PicoProbe II and Glutamate Standard: Store at -20°C. Thaw at room temperature before use. Do not keep on ice.

GLS Positive Control: Store at -20°C and always keep on ice when in use. Remove from -20°C storage immediately before use and dilute a small amount at 1:10 in the provided GLS dilution buffer. Mix by pipetting very gently. DO NOT VORTEX. DO NOT DILUTE IN GLS ASSAY BUFFER. Return the stock to -20°C immediately after preparing the working dilution.

Δ Note: *Diluting 2 µL of the GLS positive control at a time in 18 µL GLS Dilution buffer is recommended.*

Δ Note: *Keep GLS positive control,* Converter Mix A *and Developer Mix E on ice while performing the assay.*

Assay Protocol

Sample preparation:

1. Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µL GLS Assay buffer using Dounce Tissue Homogenizer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x g for 15 minutes at 4 °C.
2. Collect the supernatant (lysate) and estimate protein concentration using preferred method. We recommend BCA Protein Assay Kit II (Cat. No. ab287853). Protein concentration should range between 0.3 and 3 µg/µL.
3. Dilute sample lysates if needed using GLS Assay Buffer. For removal of small molecules that may cause high background, dilute the lysate with glutaminase assay buffer 5-10 times and filter through 10 kDa cut-off spin filters (Cat. No. ab93349). Small molecules will be removed in the ultrafiltrate, and the ultraconcentrate should be used for glutaminase activity assay. We recommend using samples for activity analysis immediately, if that is not possible; they may be stored at -80 °C for 3-4 days.
4. **Sample**: Prepare two wells for each sample labeled "Sample Background Control" (SBC), and "Sample" (S).

5. Add 2-4 µL sample (0.5 – 12 µg protein) into each of these wells. Adjust volume in each well to 50 µl with GLS Assay Buffer.
6. **For Positive Control**: add 4 - 8 µL of the GLS Positive Control (diluted in GLS dilution buffer as described above) into the desired well. Adjust volume in each well to 50 µl with GLS Assay Buffer. For Substrate Background Control: add 50 µL of GLS Assay Buffer to a well.
7. **For Substrate Background Control**: add 50 µL of GLS Assay Buffer to a well.

Δ Note: *For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.*

Glutamate Standard Curve (GSC) Generation

1. Dilute the provided Glutamate Standard 1:200 by adding 5 µL of the 0.1 M stock to 995 µL GLS assay buffer to obtain a 500 µM Standard solution.
2. Dilute the 500 µM further to obtain 100 µM solution by dissolving 100 µL of the 500 µM solution in 400 µL GLS assay buffer.
3. Add 0, 2, 4, 6, 8 and 10 µL of the 100 µM solution into a series of wells in a black 96- well plate to obtain 0, 0.2, 0.4, 0.6, 0.8 and 1 nmol/well.
4. Adjust the volume of each well to 50 µL with GLS Assay Buffer.

Reaction Mix Preparation

1. Dilute PicoProbe II (6 µL Probe + 4 µL dry DMSO).
2. Mix enough reagents for the number of assays to be performed.
3. Add GSC/SBC Mix to "Glutamate Standard Curve" wells and "Sample Background Control" wells.
4. Add Reaction Mix to Substrate Background, Sample, and Positive Control wells.
5. For each well, prepare 50 µL:

	GSC/SBC Mix	Reaction Mix
GLS Assay Buffer	44 µL	40 µL
GLS Substrate	-	4 µL
Converter Mix A	2 µL	2 µL
Developer Mix E	2 µL	2 µL
Diluted Probe	2 µL	2 µL

6. Mix well.
7. Add the reaction mix to wells of a 96-well black plate.

Δ Note: *Have the plate reader ready at Ex/Em = 535/587 nm on kinetic mode at 37°C set to record fluorescence every 30 seconds.*

Δ Note: *Prepare reaction mix immediately before adding to wells.*

Measurement

Immediately start recording fluorescence at 30 second intervals for 45-60 minutes at 37°C. Standard curve may be read in either kinetic or end point mode (after 45 minutes).

Δ Note: It is normal to observe a lag phase in the Positive Control within the first 20 min. Linear range is usually observed after 20 min of reaction.

Calculation

1. Subtract the standard background from standard RFU values, and sample background control RFU values from the sample RFU values respectively.
2. If substrate background control RFU values are higher than sample background control, subtract those values from sample RFU values instead.
3. Estimate amount of glutamate formed using the standard curve.
4. Calculate ΔM, which is the change in amount of glutamate between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction.
5. Glutaminase activity may be calculated using the following equation:

Sample Glutaminase specific activity = ΔM / (Δt x P) (nmol / (min x mg)) = mUnits / mg

Where: **ΔM =** linear change in glutamate concentration during Δt (nmol)
Δt = $t_2 - t_1$ (min)
P = sample protein content added to well (mg)

Unit Definition: One unit of glutaminase is the amount of enzyme that produces 1 µmol of glutamate per minute at pH 7 at 37°C.

Technical Support

For all technical or commercial enquiries please go to:

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