FOR RESEARCH USE ONLY!

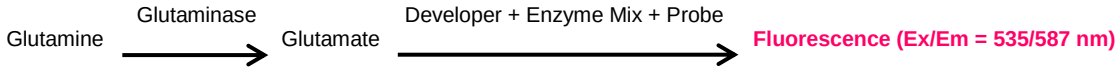
PicoProbe™ Glutaminase (GLS) Activity Assay Kit (Fluorometric)

rev 10/21

(Catalog # K455-100; 100 assays; Store at -20 ºC)

I. 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. **BioVision's PicoProbe™ Glutaminase Activity Assay Kit** 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.



II. Applications:

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

III. Sample Types:

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

IV. Kit Contents:

Components	K455-100	Cap Code	Part Number
GLS Assay Buffer	35 ml	NM	K455-100-1
GLS Dilution Buffer	200 µl	Amber	K455-100-2
GLS Substrate	2 vials	White	K455-100-3
GLS Developer	1 vial	Green	K455-100-4
GLS Enzyme Mix	1 vial	Red	K455-100-5
PicoProbe™ (in DMSO)	200 µl	Blue	K455-100-6
Glutamate Standard (0.1 M)	0.1 ml	Yellow	K455-100-7
GLS Positive Control	10 µl	Purple	K455-100-8

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multi-well spectrophotometer
- Distilled water
- Anhydrous DMSO
- 10 kDa Spin Columns (BioVision Cat# 1997)

VI. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20 °C, protected from light. Briefly centrifuge all small vials before opening. Read the entire protocol before performing the assay.

- **GLS Assay Buffer:** Warm to room temperature (RT) before use.
- **GLS Dilution Buffer:** Keep on ice, when in use.
- **GLS Substrate:** Reconstitute the GLS Substrate, one vial at a time, in 220 µl water. Heat at 37 °C in a water bath for 15-20 min to allow the substrate to dissolve complexly followed by vortexing. Divide into aliquots and store at -20 °C, protected from light. Thaw at RT before use. If precipitates are observed, heat at 37 °C in a water bath for 15 min and vortex.
- **GLS Developer and Enzyme Mix:** Reconstitute each vial with 220 µl GLS assay buffer**.** Divide into aliquots and store the remaining at -20 °C in the dark. Thaw the GLS Developer and Enzyme Mix on ice before use.
- **PicoProbe™ and Glutamate Standard:** Store at -20 °C. Thaw at RT 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.***
  Notes:
  a. We recommend diluting 2 µl of the GLS positive control at a time in 18 µl GLS Dilution buffer.
  b. Keep GLS Positive Control, GLS Developer and GLS Enzyme mix on ice while performing the assay.

VII. Glutaminase Activity Assay Protocol:

1. **Sample preparation:** Homogenize cells (4 x 10^5 cells) or tissue (10 mg) in 100 µl GLS Assay buffer using Dounce Tissue Homogenizer (BioVision Cat# 1998 or equivalent) to perform lysis and keep on ice for 10 min followed by centrifugation at 10,000 x g for 15 min at 4 °C. Collect the supernatant (lysate) and estimate the protein concentration using preferred method. We recommend BCA protein assay

kit (Biovision Cat# K813-2500). Protein concentration should range between 0.3 and 3 µg/µl. 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 (BioVision Cat# 1997). 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. **Sample:** Prepare two wells for each sample labeled **"Sample Background Control"** (SBC), and **"Sample"** (S). Add 2-4 µl sample (0.5 – 12 µg protein) into each of these wells. **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 the 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.

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

2. **Glutamate Standard Curve (GSC) Generation:** 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. 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. 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. Adjust the volume of each well to 50 µl with GLS Assay Buffer.

3. **Reaction Mix:** Dilute PicoProbe™ (6 µl Probe + 4 µl dry DMSO). Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl mix.

	GSC/SBC Mix	Reaction Mix
GLS Assay Buffer	44 µl	40 µl
GLS Substrate	-	4 µl
GLS Developer	2 µl	2 µl
GLS Enzyme Mix	2 µl	2 µl
Dilute Probe	2 µl	2 µl

Mix well and add **GSC/SBC Mix** to "Glutamate Standard Curve" wells and "Sample Background Control" wells. Add **Reaction Mix** to Substrate Background, Sample, and Positive Control wells of a 96-well black plate with flat bottom.

Notes:

a. Have the plate reader ready at Ex/Em = 535/587 nm in kinetic mode at 37 °C set to record fluorescence every 30 sec.

b. Prepare reaction mix immediately before adding to wells.

4. **Measurement:** Start recording fluorescence at 30 sec intervals for 45-60 min at 37 °C. Standard Curve may be read in either kinetic or end point mode (after 45 min). **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.

5. **Calculation:** Subtract the Standard Background from Standard RFU values, and Sample Background Control RFU values from the Sample RFU values respectively. If Substrate Background Control RFU values are higher than Sample Background Control, subtract those values from Sample RFU values instead. Estimate amount of glutamate formed using the Standard Curve. 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. Glutaminase activity may be calculated using the following equation:

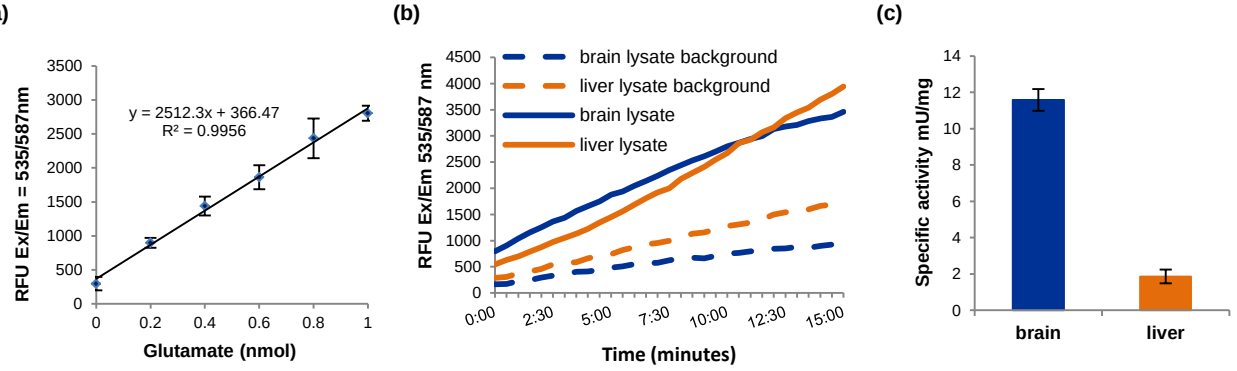
Sample Glutaminase specific activity = ΔM / (Δt x P) (nmol / (min x mg)) = mUnits / mg

Where: **ΔM =** linear change in glutamate concentration during Δt (pmol)

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.



Figures. (**a**) Glutamate Standard Curve (**b**) Enzyme kinetics for rat brain lysate (3.9 µg protein per well) and rat liver lysate (9.5 µg protein per well) **(c)** Glutaminase specific activity in rat brain (GLS1) and liver tissue (GLS2). Experiments were performed according to kit protocol.

VIII. Related Products:

Glutamate Colorimetric Assay Kit (K629)
Transglutaminase Activity Assay Kit (K571)
Transglutaminase Inhibitor Screening Assay Kit (K508)

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