MA-0164

Glutamine Assay

(100 wells, Colorimetric, OD 450 nm, Store at -20°C)

Background:

Glutamine, a non-essential amino acid, plays several important metabolic roles. Glutamine demand often outstrips production capacity and must be supplied by the diet. While important for protein synthesis, other roles are equally important. Glutamine is readily converted to glucose, particularly when intense physical activity or other stress increases glucose demand. Glutamine is both a source of nitrogen for synthesis of other molecular classes such as nucleotides and as the primary pathway for ammonia detoxification where it is used by the liver to produce urea allowing for excretion of excess amine/ammonia. Glutamine is essential for immune cells, particularly lymphocytes and macrophages, bolstering the immune response. Finally, glutamine is important to the gastrointestinal tract, providing the primary source of energy for the rapidly dividing cells of the intestinal lining. AkrivisBio's Glutamine Assay provides a simple sensitive means of quantifying glutamine from a variety of biological samples with a sensitivity in the low nanomole range.

Assay Principle:

- Glutaminase removes the amine group from glutamine sidechain forming glutamate.
- Glutamate dehydrogenase oxidatively deaminates glutamate to ketoglutarate, simultaneously reducing NAD to NADH
- NADH is used to reduce the nearly colorless.WST-8 tetrazolium to a highly colored formazan used for quantitation, measured at 450 nm.

Assay Components:

Hydrolysis Buffer	25 ml	NM	MA-0164A
Development Buffer	25 ml	WM	MA-0164B
Glutaminase	lyoph	Blue	MA-0164C
Glutamate Dehydrogenase	lyoph	Green	MA-0164D
WST-8 Reagent	lyoph	Red	MA-0164E
Glutamine Standard	lyoph	Yellow	MA-0164F

Storage Conditions and Reagent Preparation:

Store unopened kit at -20°C. Centrifuge all small vials for a few seconds prior to opening. Bring all components to room temperature before use.
Hydrolysis Buffer and Development Buffer: Store at 4°C.
Glutaminase: Reconstitute with 220 µl of Hydrolysis Buffer. Aliquot into convenient portions and store at -20°C. Keep on ice while in use.
Glutamate Dehydrogenase: Reconstitute with 220 µl Development Buffer. Aliquot to convenient portions and store at -20°C. Keep on ice while in use.
WST-8 Reagent: Reconstitute with 220 µl Development Buffer. Store at -20°C.
Glutamine Standard: Reconstitute with 100 µl DI H_2O giving a 4 mM solution. Store at -20°C.

Glutamine Assay Protocol:

1. Turn on plate reader and set temperature to 37°C.

2. Standard Curve: Dilute the Glutamine Standard to 0.4 mM by adding 10 µl to 90 µl of DI H_2O. Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of the diluted Standard to a 96-well plate to giving 0, 2, 4, 6, 8 & 10 nmol of Standard, respectively. Adjust the well volumes to 50 µl with DI H_2O.

3. Sample Preparation:

a. Homogenize tissue (10 mg) or cells (10^6) in 100 µl of Hydrolysis Buffer, on ice. Centrifuge homogenate or other biological fluids at 16,000 X g for 5 min. at 4°C. Transfer the clear supernatant to a fresh tube.

b. Deproteinize samples using trichloroacetic acid or perchloric acid procedures making sure they are properly neutralized (see PI-0102 and PI-0103) or a 10 kDa centrifugal filter. Transfer up to 50 µl to well(s) in a 96-well plate. Adjust all well volumes to 50 µl with Hydrolysis buffer..

4. Hydrolysis: Hydrolyze the samples: Add 2 µl of Glutaminase to all Standard and Sample wells

	Standard/Sample	Background Control
Hydrolysis Enzyme Mix	2 µl	---

Mix well. Incubate for 30 minutes at 37°C.

5. Reaction Mix: Each well (Standard, Sample, Background Control) requires 50 µl of Reaction Mix. Prepare sufficient material for the total number of assays to be performed containing:

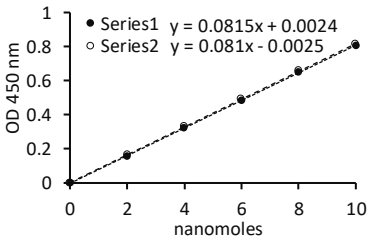
	Reaction Mix
Development Buffer	46 µl
Glutamate Dehydrogenase	2 µl
Developer	2 µl

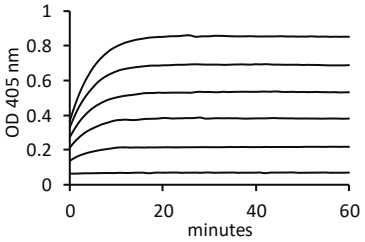
Mix well. Add 50 µl of the Reaction Mix to each well containing Standards, samples, and Background Control(s).

6. Measurement: Monitor reaction at 450 nm in a plate reader at 37°C for up to 60 minutes. Once all color development has stopped, the analysis can be terminated.

7. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0705	0.0642	0	0
2	0.2262	0.2298	0.1557	0.1656
4	0.3911	0.3940	0.3206	0.3298
6	0.5545	0.5566	0.4840	0.4924
8	0.7218	0.7232	0.6513	0.6590
10	0.8751	0.8765	0.8046	0.8123





8. Calculation: Subtract the zero Glutamine Standard reading from all other standard readings. Plot the Glutamine Standard Curve. Determine the slope of the Standard Curve. If Background Controls have been used, subtract them from their paired sample readings, otherwise subtract the zero Glutamine standard from the sample readings. Divide the Background corrected sample readings by the slope of the Standard Curve to convert well OD readings to nanomoles in the sample well. To convert back to nanomoles in the original sample:
A – Divide the nanomoles per sample well by the volume of sample added to the well in µl = nanomoles glutamine per µl sample.
B – Multiply the nanomoles glutamine per µl sample by the total volume of deproteinized supernatant recovered in step 3 above = total nanomoles glutamine per sample.
C – Divide the total nanomoles glutamine per sample by the mg tissue of # of cells = nanomoles glutamine/mg tissue (or per # of cells, etc.)

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com