Glutaminase (GLS) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: NA0754

Size:50T/24S

Components:

Extract solution: 70 mL ×1, storage at 4℃. Preheat at 37 ℃ before use.

Reagent I: powder×1, storage at 4℃. Add 1 0mL of Extract to dissolve the reagent before use.

Reagent IIA: 1 mL×1, storage at 4℃.

Reagent IIA: 4 mL×1, storage at 4℃. Before use, pour Reagent II A into Reagent II B to mix (A:B=1:4 ratio), or prepare according to the volume ratio Reagent IIA : Reagent II B = 1:4 before use.

Reagent III: 5 mL×1, storage at room temperature.

Standard: 1 mL ×1, storage at 4℃. 10 μmol/mL nitrogen standard solution. Preheat at 37℃ before use.

Product Description:

GLS (EC3.5.1.2) is mainly found in higher animals and some bacteria and plant roots, catalyzing the hydrolysis of glutamine into glutamic acid and ammonia, which plays an important role in the regulation of nitrogen metabolism, especially the regulation of free ammonia and urea metabolism.

The kit uses the indophenol blue colorimetric method to determine ammonia produced by glutamine of GLS-catalyzed to indicate activity

Reagents and Equipment Required but Not Provided:

Spectrophotometer, adjustable pipette, mortar/homogenizer, centrifuge, 1 mL glass cuvette, ice and distilled water.

Sample preparation:

1. Tissues: The mass (g): volume of distilled water(mL)= 1:5-10, suggested 0.1g of tissues, add 1 mL of Extract solution and fully grind. Centrifuge at 12000g at 4 °C for 15 min, then take supernatant on ice to be tested.
2. Bacteria or cells

   Accordance ratio bacteria or cell amount (10^4): volume of Extract solution (mL)=500~1000:1. Suggested 5 million with 1 mL of Extract solution. Use ultrasonic to splitting bacteria or cell (placed on ice, powder: 300W, work time 3s，interval 7s，total time 3 min). Centrifuge at 12000g at 4°C for 15 min. then take supernatant on ice to be tested.

Procedure:

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 630 nm and set the counter to zero with distilled water.

2. Dilute the standard solution 32 times with the Extract solution to obtain the standard solution of 0.3125 μmol/mL.
3. Add reagent to a EP tube:

Reagent name (mL)	Test tube (At)	Control tube (Ac)	Standard tube (As)	Blank tube (Ab)
Sample	80	80	-	-
Extract	-	320	-	400
Reagent I	320	-	-	-
Mix and react for 60 min at 37℃			-	-
Standard	-	-	400	-
Reagent II	80	80	80	80
Reagent III	60	60	60	60
Distilled water	460	460	460	460

Mix well, react for 30min at room temperature. Measure the absorbance at 630nm. Recorded as At, Ac, As, Ab. Calculate ΔAs = As - Ab, ΔAt = At - Ac.

Calculation:

1. Protein concentration

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of NH_3-N per hour every milligram of tissue protein.

   GLS（U/mg prot）=ΔAt÷（ΔAs÷Cst）×Ver÷(Vsa×Cpr)÷T=1.5625×ΔAt÷ΔAs÷Cpr。
2. Fresh weight

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of NH_3-N per hour every gram of sample.

   GLS (U/g) =ΔAt÷（ΔAs÷Cst）×Ver÷(W÷Ve×Vsa)÷T=1.5625×ΔA÷ΔAs÷W
3. Number of cells

   Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1μmol of NH_3-N per hour every 5×10^4 cells.

   GLS (U/mL) =ΔAt÷（ΔAs÷Cst）×Ver÷(Vsa ÷Ve)÷T=1.5625×ΔA÷ΔAs

   Cst: Standard solution concentration,0.3125 μmol/mL;

   Vsa: Supernatant volume added, 0.08 mL;

   Ver: Volume of enzymatic reaction, 0.4 mL;

   Ve: Volume of add Extract solution, 1 mL;

   Cpr: Sample protein concentration, mg/mL;

   W: Fresh weight of sample, g;

   T: React time, 1 hour.

Note:

1. If OD>0.7, It is recommended to further dilute the supernatant and then measure it. Multiply the dilution ratio in calculation.

2. Reagent II should be used as soon as possible. If discoloration is found, it can no longer be used.

Recent Product citations：

[1] Fu Y, Lei F, Wang J, et al. Maternal Cigarette Smoke Exposure Disturbs Glutamate/GABA Balance in pFRG of Neonatal Rats[J]. Respiratory Physiology & Neurobiology, 2020: 103383.

[2] Liu S, Li N, Lin Q, et al. Glutaminase 1 in mandarin fish Siniperca chuatsi: Molecular characterization, expression pattern and function involving in virus replication[J]. Aquaculture, 2020: 734924.

References：

[1] Mahajan R V, Saran S, Kameswaran K, et al. Efficient production of L-asparaginase from Bacillus licheniformis with low-glutaminase activity: optimization, scale up and acrylamide degradation studies[J]. Bioresource technology, 2012, 125: 11-16.

Related Products:

NA0865/NA0622 Nitrate reductase（NR）Activity Assay kit
NA0754/NA0512 Glutaminase (GLS) Assay Kit
NA0751/NA0509 Nitrite Assay Kit (Water And Soil)