Product Manual

PQQ Assay Kit

Catalog Number

MET-5213 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Pyrroloquinoline quinone (PQQ) is an essential cofactor that binds to proteins in all organisms. The cofactor is produced in certain types of bacteria and is often acquired through ingestion through food or supplements containing PQQ. It plays a role in energy metabolism and is also thought to help in mitochondrial health as an antioxidant, fight inflammation, and have antidiabetic properties.

Cell Biolabs' PQQ Assay Kit is a simple colorimetric assay that measures PQQ present in biological samples such as cell lysates or tissue extracts in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, PQQ standards and unknown samples. The total PQQ concentrations of unknown samples are determined by comparison with a known PQQ standard. The kit has a detection sensitivity limit of approximately 0.15 ng/ml PQQ.

Assay Principle

Cell Biolabs' PQQ Assay Kit is a convenient quantitative tool that measures PQQ within biological samples. The assay is based on an enzymatic activity reaction of glucose dehydrogenase (GDH). *E. coli* GDH lacks activity unless PQQ is present. Active GDH results in an electron transfer to a colorimetric probe that produces a colored product that can be measured at 450 nm. The intensity of the product color is proportional to the amount of PQQ within a sample. Samples are compared to a known concentration of PQQ standard within the 96-well microtiter plate format (Figure 1).

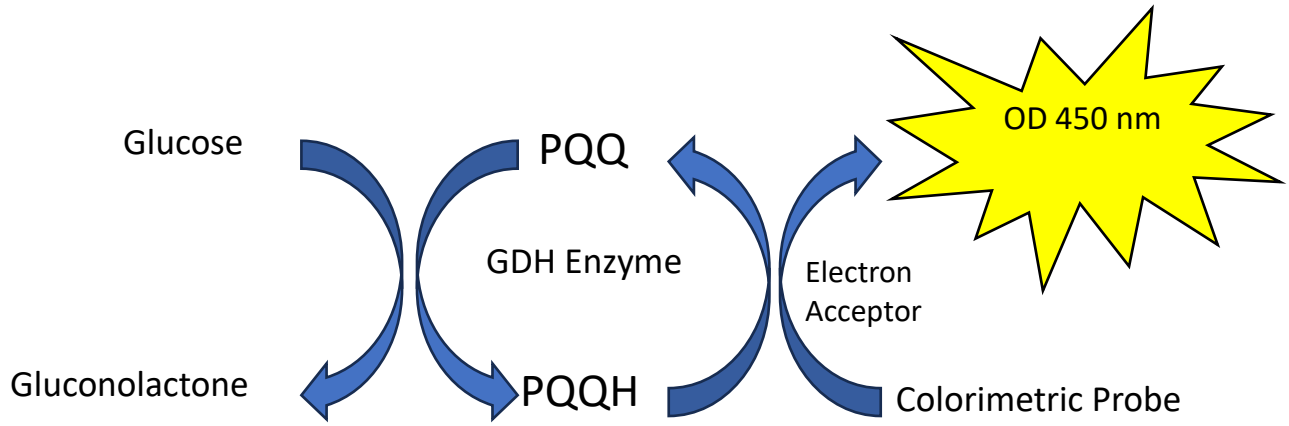

Figure 1. PQQ Assay Principle.

Related Products

1. MET-5018: $NADP^+$/NADPH Assay Kit
2. MET-5163: ATP Assay Kit
3. STA-680: Glucose Assay Kit (Colorimetric)
4. MET-5012: Lactate Assay Kit (Colorimetric)
5. MET-5080: Glutamate Assay Kit (Colorimetric)

Kit Components (shipped on dry ice)

1. Colorimetric Probe (5X) (Part No. 52131C): One 2 mL amber tube
2. Electron Acceptor (20X) (Part No. 52132C): One 500 µL tube
3. PQQ Standard (Part No. 52133C): One 50 µL tube of 1 µg/mL PQQ
4. GDH Enzyme (Part No. 52134D): One 5 mL bottle
5. Assay Buffer (10X) (Part No. 52135B): One 1 mL tube

Materials Not Supplied

Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

Storage

Upon receipt, store the GDH Enzyme at -80°C. Store the Colorimetric Probe (5X), Electron Acceptor (20X), and PQQ Standard at -20°C. Store the remaining components at 4°C.

Preparation of Reagents

- GDH Enzyme: Avoid repeated freeze thaws. Aliquot reagent into smaller portions and freeze at -80ºC.
- Reaction Buffer: Prepare a Reaction Buffer for the number of assays being tested and just before use. Prepare by diluting the Colorimetric Probe (5X) 1:5, Electron Acceptor (20X) 1:20, and Assay Buffer (10X) 1:10 in deionized water (e.g. For 10 assays, combine 200 µL of Colorimetric Probe (5X), 50 µL of Electron Acceptor (20X), 100 µL of Assay Buffer (10X) with 650 µL of deionized water for a total of 1 mL solution). Use the Reaction Buffer immediately for the assay.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. It is highly recommended that all samples should be assayed immediately upon preparation or stored for up to 1 month at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in deionized water as necessary before testing. Run proper controls and account for any sample dilutions. Always run a standard curve with samples.

- Tissue homogenates: Sonicate or homogenize 100 mg tissue sample in 0.5 mL cold 1X PBS Buffer. Centrifuge at 14,000 rpm for 5 minutes at 4°C to remove insoluble material. Perform dilutions in cold 1X PBS. Sample may be tested immediately. Store unused samples at -80°C for up to 1 month.

- Cell lysates: Culture cells until confluent and harvest. Centrifuge and wash cell pellet with 1X PBS. Centrifuge to pellet cells and remove wash. Resuspend cells at 1-5 x 10^6 cells/mL in 0.5 mL 1X PBS Buffer. Sonicate the cells on ice. Centrifuge at 14,000 rpm for 5 minutes 4°C to remove cell debris. Perform dilutions in 1X PBS. Sample may be tested immediately. Store unused samples at -80°C for up to 1 month.

Note: PQQ is sensitive to heat. Avoid high temperatures (>25°C) for an extended amount of time.

Preparation of Standard Curve

Immediately before use, prepare a dilution series of PQQ standards in the concentration range of 0 to 10 ng/mL according to Table 1. Vortex thoroughly. Do not store standard solutions.

Standard Tubes	1 µg/mL PQQ Standard (µL)	Deionized Water (µL)	PQQ (ng/ml)
1	5	495	10
2	250 of Tube #1	250	5
3	250 of Tube #2	250	2.5
4	250 of Tube #3	250	1.25
5	250 of Tube #4	250	0.625
6	250 of Tube #5	250	0.313
7	250 of Tube #6	250	0.156
8	0	250	0

Table 1. Preparation of PQQ Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.
2. Add 50 µL of each PQQ standard (Table 1) or unknown samples into wells of a 96-well microtiter plate.
3. Add 50 µL of GDH Enzyme to each well. Mix the well contents thoroughly and incubate for 30 minutes at room temperature with agitation.
4. Add 100 µL of Reaction Buffer to each well. Mix the well contents thoroughly. Protect from light and incubate for 2 hours at room temperature with agitation.

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics up to 24 hours.
5. Read the plate with a spectrophotometric microplate reader at 450 nm.
6. Calculate the concentration of PQQ within samples by comparing the sample OD to the standard curve.

Example of Results

The following figures demonstrate typical PQQ Assay Kit results. One should use the data below for reference only. This data should not be used to interpret or calculate actual sample results.

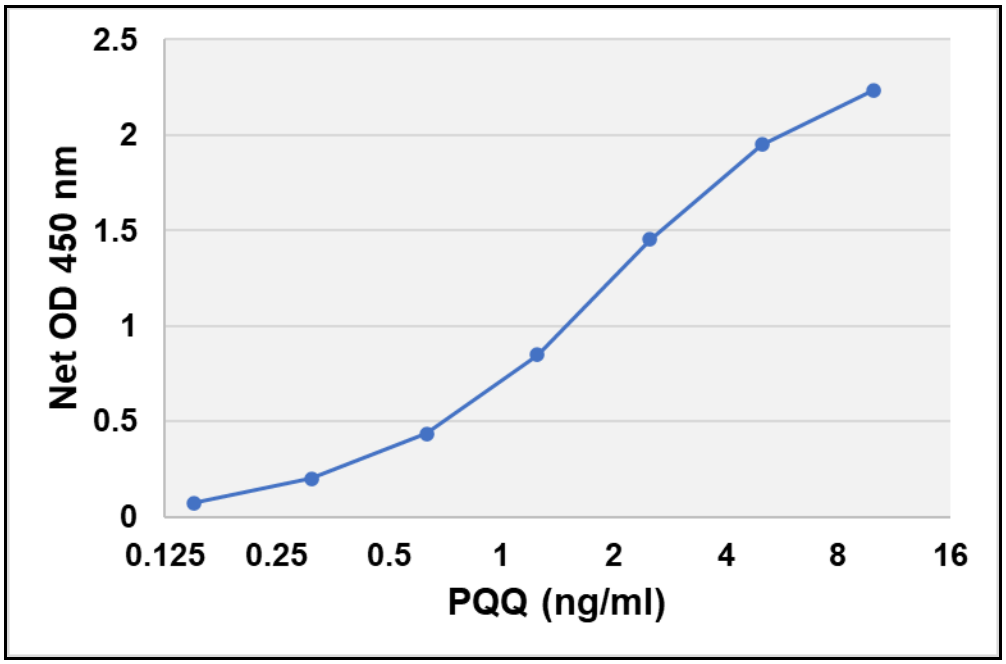

Figure 2: PQQ Standard Curve.

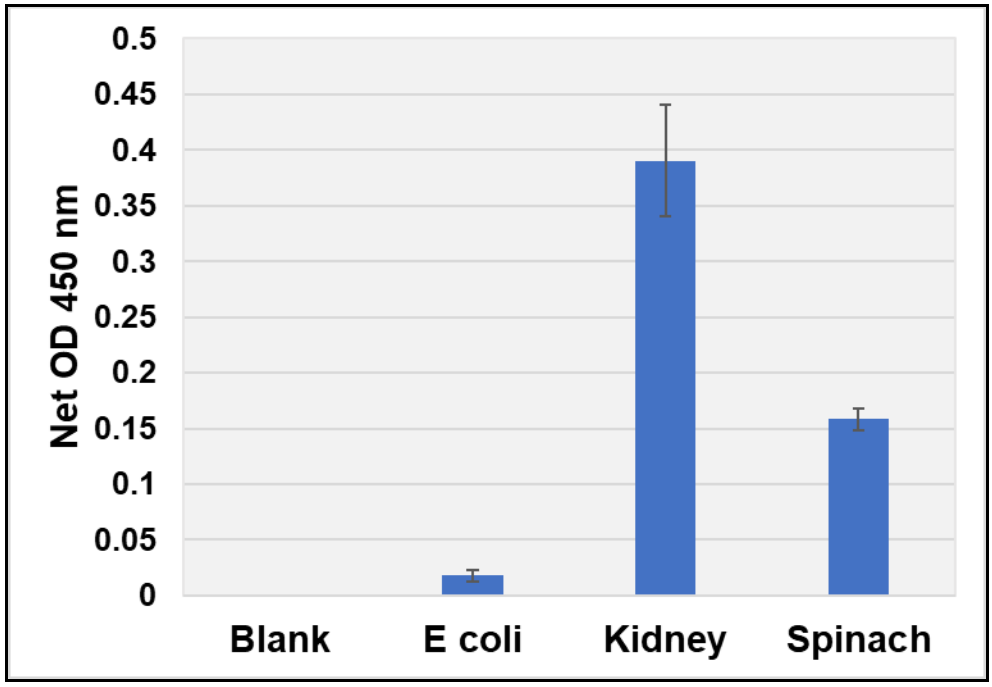

Figure 3: PQQ detection in pig kidney and spinach. PQQ in samples were tested according to the Assay Protocol of PQQ Assay Kit.

References

1. Kumazawa T, Sato K, Seno H, Ishii A, Suzuki O (1995). *Biochem. J.* **307**: 331–333.
2. Matsushita K, Ameyama M (1982). *Methods Enzym.* **89**: 149–154.
3. Matsushita K, Arents, JC, Bader, R, Yamada, M, Adach O, Postma PW (1997). *Microbiol.* **143**: 3149–3156

4. Kato C, Kawai E, Shimuzu N, Mikekado T, Kimura F, Miyazawa T, Nakagawa K. (2018). *PLoS ONE*. **13**: e0209700.

Warranty

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