Phosphoenolpyruvate Assay Kit

ARG83606 Phosphoenolpyruvate Assay Kit is a detection kit for the quantification of Phosphoenolpyruvate.

Catalog number: ARG83606

Package: 100 assays

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PRINCIPLE OF THE ASSAY

ARG83606 Phosphoenolpyruvate Assay Kit measures total PEP within biological samples. PEP in the presence of ADP is converted by Pyruvate Kinase to pyruvate + adenosine triphosphate (ATP). Pyruvate is converted by pyruvate oxidase in the presence of phosphate and oxygen into acetyl phosphate, carbon dioxide, and hydrogen peroxide. The resulting hydrogen peroxide is then detected with a highly specific fluorometric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples are compared to a known concentration of PEP standard within the 96-well microtiter plate format. Samples and standards are incubated for 30 minutes and then read with a standard 96-well fluorometric plate reader.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store **10x Assay Buffer** and **$MgCl_2$** at **RT**, **Pyruvate Kinase** at **-80°C**, **other component** store at **-20°C**. Use the kit before expiration date.

Component	Quantity	Storage information
Phosphoenolpyruvate Standard (20 mM Phosphoenolpyruvate)	50 µl	-20°C
Aenosine diphosphate (ADP)	250 µl	-20°C
Probe	50 µl	-20°C
HRP	10 µl	-20°C
Flavin Adenine Dinucleotide (FAD)	50 µl	-20°C
Thiamine Pyrophosphate (TPP)	50 µl	-20°C
Pyruvate Oxidase	300 µl	-20°C

Pyruvate Kinase	200 µl	-80°C
10X Assay Buffer	25 ml	RT
Magnesium Chloride ($MgCl_2$)	200 µl	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Black microplate reader
- Pipettes and pipette tips
- Deionized or distilled water
- Centrifuge spin filter

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid sample.
- Upon receipt, store **10x Assay Buffer** and **$MgCl_2$** at **RT**, **Pyruvate Kinase** at **-80°C**, **other component** store at **-20°C**. Use the kit before expiration date.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or sample.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants- Remove particulates by centrifugation for 10 min at 1500 x g at 4°C and aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Urine- Collect the first urine of the day, micturate directly into a sterile container. Remove impurities by centrifugation. Collect the supernatants and assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Cell or Tissue Lysate- Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect samples and assay immediately or aliquot and store samples at-80°C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1x Assay Buffer** - Dilute the **10x Assay Buffer** into Deionized Water to yield **1x Assay Buffer**. The **1x Assay Buffer** is stable for up to 6 months at 2-8°C.

- **Reaction / Negative Control Mix**: Prepare as below:

Component	Reaction Mix	Negative Control Mix
Pyruvate Kinase	100 µl	---
$MgCl_2$	100 µl	100 µl
ADP	125 µl	125 µl
HRP	5 µl	5 µl
Pyruvate Oxidase	150 µl	150 µl
FAD	25 µl	25 µl
TPP	25 µl	25 µl
Probe	25 µl	25 µl
1XAssay Buffer	1945 µl	2045 µl
Total	2500 µl	2500 µl

- **Standards:** Prepare fresh Lysine Standards before use by diluting in 1X Assay Buffer according to the Table below.

Standard tube	Phosphoenolpyruvate (µM)	1X Assay Buffer (µL)	Standard (µL)
S1	200	495	5 (20 mM Phosphoenolpyruvate)
S2	100	250	250 of S1
S3	50	250	250 of S2
S4	25	250	250 of S3
S5	12.5	250	250 of S4
S6	6.25	250	250 of S5
S7	3.13	250	250 of S6
S0	0	200	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use. Standards and sample should be assayed in duplicates.

1. Add 50 µl of diluted sample or each diluted Standard into respective wells of the 96-well plate.
2. Add 50 µl of Reaction / Negative Control Mix to each well.
3. Cover the plate and incubate for 30 minutes at 37°C.
4. Read the absorbance with a plate reader at O.D. 530-570 nm.

CALCULATION OF RESULTS

Plot the RFU measured at 15 minutes for each standard against the standard concentrations. Determine the slope using linear regression fitting. The Phosphoenolpyruvate concentration of a sample is calculated as follow:

Net RFU = (RFU_{+PK})- (RFU_{-PK})

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the Phosphoenolpyruvate. One should use the data below for demonstration only and cannot be used in place of data generations at the time of assay.