Technical Bulletin

Pyruvate Kinase Assay Kit

Catalogue number MAK485

Product Description

Pyruvate Kinase (PK) is an enzyme involved in glycolysis. It catalyzes the transfer of a phosphate group from phosphor(enol)pyruvate (PEP) to ADP, yielding one molecule of pyruvate and one molecule of ATP. Pyruvate kinase deficiency, a genetic disease, is caused by a lack of pyruvate kinase and slows down the process of glycolysis. Pyruvate kinase is also involved in gluconeogenesis, a biochemical pathway in which the liver generates glucose from pyruvate and other substrates.

The Pyruvate Kinase Assay Kit provides a simple, direct, and automation-ready procedure for measuring pyruvate kinase activity. In this assay PEP and ADP are catalyzed by pyruvate kinase to generate pyruvate and ATP. The color intensity of the reaction product at 570 nm or fluorescence intensity at λ_{Ex}=530 nm/λ_{Em}=590 nm is directly proportional to the pyruvate generated by the pyruvate kinase in the sample.

The linear detection range of the is 0.1-50units per liter (U/L) pyruvate kinase for the colorimetric assay and 0.01-2 U/L for the fluorometric assay. The kit is suitable for pyruvate kinase activity determination in plasma, serum, and tissue samples, as well as for studying the effects of drugs on pyruvate kinase activity.

Components

The kit is sufficient for 100 Colorimetric/Fluorometric assays in 96-well plates.

- Developer 12 mL
  Catalogue Number MAK485A
- Cosubstrate 120 µL
  Catalogue Number MAK485B
- Dye Reagent 120 µL
  Catalogue Number MAK485C
- Pyruvate Standard (25 mM) 400 µL
  Catalogue Number MAK485D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Multiwell plate reader
- Clear flat-bottom 96-well plates for colorimetric assay or black flat-bottom 96-well plates for fluorometric assay. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set.
  (Catalogue Number D9063 or equivalent)
- Microcentrifuge capable of RCF ≥ 14,000 ×*g*
- Phosphate Buffered Saline (PBS)
  (Catalogue Number P3813 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Developer: Equilibrate Developer to desired assay temperature prior to use.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum

Serum samples should be diluted at least 4-fold in purified water.

Tissue and Cells

Homogenize tissue (20 mg) or cells (2×10^6) in 100 µL of cold 1× PBS and then centrifuge for 5 minutes at room temperature at 14,000 × *g* to pellet any debris. Use the clear supernatant for the assay.

All Samples

Transfer 10 µL of Sample to wells of a 96-well plate.

Colorimetric Standard Curve Preparation

1. Prepare a 1000 µM Pyruvate Standard by mixing 20 µL of the 25 mM Pyruvate Standard and 480 µL of purified water.
2. Prepare Pyruvate standards in 1.5 mL microcentrifuge tubes according to Table 1.

Table 1.

Preparation of Colorimetric Pyruvate Standards

Well	1000 µM Standard	Purified Water	Pyruvate (µM)
1	200 µL	-	1000
2	120 µL	80 µL	600
3	60 µL	140 µL	300
4	-	200 µL	0

3. Mix well and transfer 10 µL of each Standard into separate wells of a clear 96-well plate.

Fluorometric Standard Curve Preparation

1. Prepare Pyruvate standards according to Colorimetric Standard Curve Preparation section.
2. Further dilute 20 µL of each of the Standards from Colorimetric Procedure with 180 µL of purified water according to Table 2.

Table 2.

Preparation of Fluorometric Pyruvate Standards

Well	Colorimetric Standard	Purified Water	Pyruvate (µM)
1	10 µL of 1000 µM Std	190 µL	50
2	10 µL of 600 µM Std	190 µL	30
3	10 µL of 300 µM Std	190 µL	15
4	-	200 µL	0

1. Mix well and transfer 10 µL of each Standard into separate wells of a black 96-well plate.

Working Reagent

This assay is based on an enzyme-catalyzed kinetic reaction. To ensure identical incubation time, addition of Working Reagent should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 97 µL of Working Reagent according to Table 3.

Table 3.

Preparation of Working Reagent

Reagent	Volume
Developer	95 µL
Cosubstrate	1 µL
Dye Reagent	1 µL

2. Add 90 µL of Working Reagent to each Standard and Sample well. Tap plate to mix.

Measurement

1. Incubate at room temperature or desired reaction temperature for 5 minutes. Protect plate from light for fluorometric assay. Measure the optical density (OD) at 570nm or fluorescence intensity (F) at λ_{Ex}=530 nm and λ_{Em}=590 nm at time 5 minutes (R_5).
2. Incubate the plate for an additional 30 minutes at room temperature or desired reaction temperature.
3. Measure the OD at 570 nm or F at λ_{Ex}=530nm/λ_{Em}=590 nm at time 35 minutes (R_{35}). Alternatively, monitor the optical density or fluorescence intensity of the plate for 35 minutes in kinetic mode and record the 5-minute and 35-minute readings.

Results

1. Use optical density or fluorescence intensity measured at 35 minutes (R_{35}) to determine the slope of the standard curve.
2. Determine ΔOD or ΔF by subtracting the Blank value (Standard #4) from the remaining standard values.
3. Plot the ΔOD or ΔF against standard concentrations and determine the slope by linear regression.
4. To determine the pyruvate kinase activity in the Sample, first calculate ΔR by subtracting the R_5 from R_{35} for each Sample and Standard#4 (Blank).
5. Calculate the pyruvate kinase (PK) activity as follows:
   Pyruvate Kinase (U/L)

$$\frac{\Delta R_{Sample} - \Delta R_{Blank}}{Slope\ (\mu M^{-1}) \times T} \times DF$$

where:

ΔR_{Sample} = Change in optical density (OD) or fluorescence intensity (F) reading of Sample

ΔR_{Blank} = Change in optical density (OD) or fluorescence intensity (F) reading of Blank (Standard #4)

T = Time of reaction (30 minutes)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

If the calculated PK activity is higher than 50 U/L for the colorimetric assay or higher than 2 U/L for the fluorometric assay, dilute the sample in purified waterand repeat assay. Multiply result by the dilution factor.

Unit definition: One unit of PK will generate 1 µmole of pyruvate and 1 µmole ATP from PEP and ADP per minute at 25 °C at pH 7.5.

Figure 1.
Typical Colorimetric Pyruvate Kinase Titration (30-minute reaction at 25 °C)

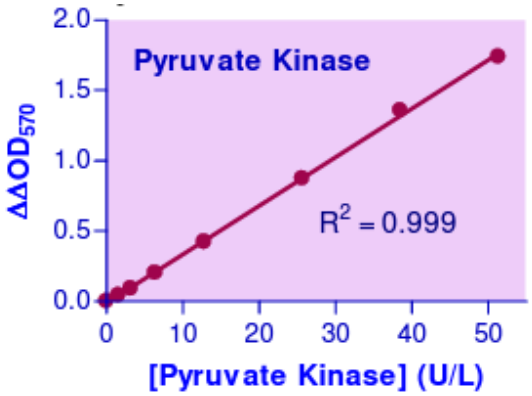
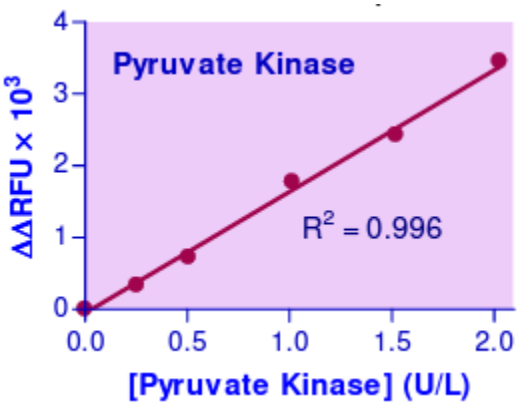


Figure 2.
Typical Fluorometric Pyruvate Kinase Titration (30-minute reaction at 25 °C)



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