ab83432 - Pyruvate Kinase (PK) Assay Kit

For the rapid, sensitive and accurate measurement of Pyruvate Kinase (PK) activity in various samples.
This product is for research use only and is not intended for diagnostic use.

Kit components

Item	Quantity	Storage Temperature (before prep)
PK Assay Buffer	25 mL	-20°C or 4°C
OxiRed™ Probe	0.2 mL	-20°C
Developer Mix A	1 vial	-20°C
PK Substrate Mix	1 vial	-20°C
PK Positive Control	1 vial	-20°C
Pyruvate Standard	100 µL	-20°C

PLEASE NOTE: Developer Mix A was previously labelled as Development Enzyme Mix I and PK Enzyme Mix (Lyophilized). The composition has not changed.

Storage

All components in this kit are shipped on blue ice and are suitable for storage at -20°C, unless reconstituted. Upon receipt, immediately store kit at -20°C in the dark. Individual components may be stored at alternative temperatures as shown in the components table.

Oxired™ Probe: Ready to use as supplied. Warm the DMSO solution at 37 °C until completely thawed (~ 5 minutes), vortex briefly to create a homogenous solution, and spin down briefly to collect any contents trapped in the cap or seal. Keep at room temperature during use and reseal promptly to minimize moisture exposure.
For multiple use aliquot and store at −20 °C, protected from light and moisture. Stable for up to 2 months after thawing (≤3 freeze–thaw cycles).
PK Substrate Mix and Developer Mix A: Dissolve with 220 µL dH_2O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.
PK positive control: Dissolve with 100 µL diH_2O. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.
PK Assay Buffer: Store at +4°C.

Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent or colorimetric microplate reader
- 96 well clear plate (for colorimetric assay) and 96 well black plate (for fluorometric assay)
- Orbital shaker

Assay Protocol

1. Sample Preparation:

Biological fluids can be directly added into sample wells. Tissues or cells can be extracted with 4 volumes of the Assay Buffer, centrifuge to get clear extract, and assay sample immediately. Add samples directly into 96-well plate, bring volume to 50 µL/well with PK Assay Buffer.
Recommended dilutions:
Colorimetric assay: Cell samples: 10-50 fold. Tissue samples: 30-100 fold.
Fluorometric assay: Biological fluids 50-100 fold.
We suggest testing several doses of your sample to ensure the readings are within the linear range.
For the **positive control** (optional), perform a 1:10 dilution by adding 10 µL of positive control into 90 µL of PK assay buffer, then add 5 µL positive control solution to wells (use 0.5-2 µL Positive Control for fluorometric assay), adjust volume to 50 µL/well with Assay Buffer.

2. Standard Curve Preparation:

a. For the colorimetric assay:

Dilute the Pyruvate Standard to 1 nmol/µL by adding 10 µL of the Standard to 990 µL of Assay Buffer, mix well.
Add 0, 2, 4, 6, 8, 10 µLof the diluted standard into a series of wells. Adjust volume to 50 µL/well with Assay Buffer to generate 0, 2, 4, 6, 8, and 10 nmol/well of the Pyruvate Standard.

b. For the fluorometric assay:

Dilute the Pyruvate Standard to 1 nmol/µL as for the colorimetric assay. Then dilute the standard another 10-fold to 0.1 nmol/µl by mixing 10 µL with 90 µL of Pyruvate Assay Buffer. Mix well.
Add 0, 2, 4, 6, 8, 10 µL of the diluted standard into a series of wells. Adjust volume to 50 µL/well with Assay Buffer to generate 0, 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/well of the Pyruvate Standard.

3. Reaction Mix Preparation:

Mix enough reagents for the number of standard and assays to be performed. For each well, prepare a total 50 µL Reaction Mix containing:

	Pyruvate Kinase Measurement	Background Control*
Assay Buffer	44 µL	46 µL
Substrate Mix	2 µL	---
Developer Mix A	2 µL	2 µL
OxiRed™ Probe	2 µL	2 µL

***Note:** Pyruvate in the sample will generate background. If significant amount of pyruvate is in your sample, the background control should be performed. The background readings are then subtracted from your sample readings.
The fluorometric assay is ~10 times more sensitive than the colorimetric assay. Use 0.4 µl of the probe per reaction to decrease the background reading/increase detection sensitivity significantly.

4. Add 50 µL of the reaction mix to each well containing the pyruvate standard, samples and controls, mix well.
5. Measure OD_{570nm} or fluorescence Ex/Em = 535/587 nm at T_1 to read A_1, measure again at T_2 after incubating the reaction at 25°C for 10-20 min (or incubate longer time if the PK activity is low in sample) to read A_2, protect from light. The signal increase is due to pyruvate generated by PK, $\mathbf{\Delta A = A_2 - A_1}$.

Note:
It is essential to read A_1 and A_2 in the reaction linear range. It will be more accurate if you read the reaction kinetics. Then choose A_1 and A_2 in the reaction linear range.

Data Analysis

Subtract zero standard readings from the standards.
Plot the pyruvate standard curve. Apply the ΔA to the standard curve to get B nmol of pyruvate generated between T_1 and T_2 by PK in the reaction wells.

PK calculation:

$$\text{PK Activity} = \frac{\text{B x Sample Dilution Factor}}{(T_2 - T_1) \times V} = \text{nmol/min/ml = mU/mL}$$

Where:
B is the pyruvate amount from pyruvate standard curve (in nmol).
T_1 is the time of the first reading (A_1) (in min).
T_2 is the time of the second reading (A_2) (in min).
V is the sample volume added into the reaction well (in mL).
Unit definition: One unit of Pyruvate Kinase is the amount of enzyme that will transfer a phosphate group from PEP to ADP, yielding 1.0 µmol of pyruvate per minute at 25°C.

Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range
Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the **10kDa spin column (ab93349)**
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use

Problem	Reason	Solution
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended

		concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

Technical Support

For all technical or commercial enquiries please go to:
https://www.abcam.com/en-us/contact-us
https://www.abcam.cn/contact-us (China)
https://www.abcam.co.jp/contact-us (Japan)