DATA SHEET

For research use only

Pyruvate Assay Kit

(Catalog #K609-100; 100 assays; Store kit at –20˚C)

I. Introduction:

Pyruvate is a central molecule in metabolism through which sugars enter the citric acid cycle. Pyruvate can be converted to carbohydrates during gluconeogenesis or to fatty acids via acetyl CoA. High levels of pyruvate are associated with liver disease and genetic disorders. Pyruvate has also been used to stimulate metabolism leading to loss of body weight. BioVision provides a simple, direct and automation-ready procedure for measuring pyruvate concentration in various biological samples such as blood, cells, culture and fermentation media, etc. In the assay, pyruvate is oxidized by pyruvate oxidase via enzyme reactions to generate color (λ = 570 nm) and fluorescence (at Ex/Em = 535/587 nm). Since the color or fluorescence intensity is proportional to pyruvate content, the pyruvate concentration can be accurately measured. The kit detects 1 µM to 10 mM pyruvate.

II. Kit Contents:

Components	100 assays	Cap Color	Part Number
Pyruvate Assay Buffer	25 ml	WM	K609-100-1
Pyruvate Probe (in DMSO)	200 µl	Red	K609-100-2A
Pyruvate Enzyme Mix	Lyophilized	Green	K609-100-4
Pyruvate Standard (100 nmol/µl)	100 µl	Yellow	K609-100-5

III. Reagent Preparation and Storage Conditions:

Pyruvate Probe: Briefly warm at 37˚C for 1-2 min to completely melt the DMSO solution. Mix well, store at –20˚C, protected from light and moisture. Use within two months.

Pyruvate Enzyme Mix: Dissolve with 220 µl Pyruvate Assay Buffer. Pipette up and down to completely dissolve. Store at –20˚C. Use within two months.

IV. Pyruvate Assay Protocol:

1. **Standard Curve Preparations:**

Colorimetric assay: Dilute the Pyruvate Standard to 1 nmol/µl by adding 10 µl of the Standard to 990 µl of Pyruvate Assay Buffer, mix well.

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 taking 10 µl into 90 µl of Pyruvate Assay Buffer. Mix well.

Add 0, 2, 4, 6, 8, 10 µl into a series of standards wells. Adjust volume to 50 µl/well with Pyruvate Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Pyruvate Standard for the colorimetric assay (0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well for the fluorometric assay).

2. **Sample Preparations:** Prepare test samples in 50 µl/well with Pyruvate Assay Buffer in a 96-well plate. Serum can be directly added into sample wells, and adjust volume to 50 µl/well with Pyruvate Assay Buffer (serum contains ~50-100 pmol/µl pyruvate). Tissues or cells can be extracted with 4 volume of the Pyruvate Assay Buffer, centrifuge to get clear pyruvate extract. We suggest using several doses of your sample to ensure the readings are within the standard curve range. Due to the presence of LDH in serum, care must be taken during sample processing to prevent the conversion of pyruvate to lactate. Samples can be deproteinized by 10Kd cutoff spin filter (BioVision Cat #1997-25) to remove proteins.

3. **Reaction Mix Preparation:** Mix enough reagents for the number of assays performed. For each well, prepare a total 50 µl Reaction Mix containing the following components. Mix well before use:

46 µl Pyruvate Assay Buffer
2 µl Pyruvate Probe
2 µl Enzyme Mix

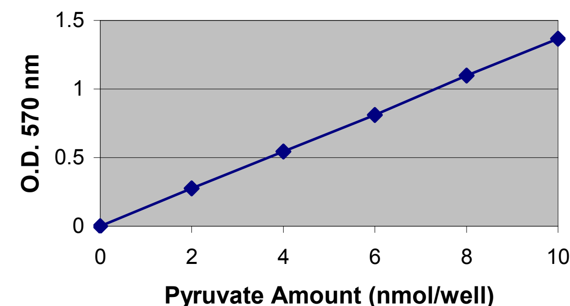
Note (optional): for fluorometric assay use 0.4 µl Pyruvate Probe and 47.6 µl Pyruvate assay buffer to reduce background

4. Add 50 µl of the Reaction Mix to each well containing the Pyruvate Standard or test samples, mix well.
5. Incubate the reaction for 30 minutes at room temperature, protect from light.
6. Measure O.D. 570 nm for colorimetric assay or fluorescence at Ex/Em = 535/590 nm in a microplate reader.
7. **Calculation:** Correct background by subtracting the value derived from the 0 pyruvate control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot standard curve nmol/well vs. O.D. 570 nm readings. Then apply the sample readings to the standard curve to get pyruvate amount in the sample wells (Py).

The pyruvate concentrations in the test samples:

C = Py/Sv (nmol/µl or mM)

Where: Py is the amount of pyruvate (nmol) of your sample from standard curve.
Sv is the sample volume (µl) added into the sample well.
Pyruvate molecular weight: 88.08. Pyruvate concentration in your sample can be expressed as nmol/ml, or mg/ml, or mg/dL or mM (mmol/liter).
1 mM = 8.81 mg/dL.



RELATED PRODUCTS:

- Lactate Assay Kit
- Cholesterol Assay Kit
- Glutathione Assay Kit
- Glucose, Sucrose Assay Kit
- Maltose Assay Kit
- Ascorbic acid Assay Kit
- Free Fatty Acid Assay Kit
- NAD(P)/NAD(P)H Assay Kit
- ATP/ADP Assay Kit
- Cell Proliferation Assay Kit
- Cytotoxicity Assay Kit

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate	• Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples	• Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used	• Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots	• Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range	• Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		