Ab65342 Pyruvate Assay Kit (Colorimetric/Fluorometric)

For the rapid, sensitive and accurate measurement of pyruvate levels in various samples.

This product is for research use only and is not intended for diagnostic use

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols

Storage and stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

Materials supplied

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer IV/Pyruvate Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Pyruvate Probe (in DMSO)	200 µL	-20°C	-20°C
Development Enzyme Mix I/Pyruvate Enzyme Mix	1 vial	-20°C	-20°C
Pyruvate Standard/Pyruvate Standard (100 nmol/µL)	100 µL	-20°C	-20°C

Materials required, not supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric or fluorescent microplate reader – equipped with filter for OD 570 nm or Ex/Em = 535/587 nm (respectively)
- 96 well plate: black plates (clear bottoms) for fluorometric assay; clear plates for colorimetric assay
- Orbital shaker
- Dounce homogenizer (if using cells or tissue)

If performing deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium hydroxide (KOH), 2M
- Ice

Quick assay procedure

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, OxiRed Probe/Pyruvate probe and enzyme mix (aliquot if necessary); get equipment ready
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare Reaction Mix (Number samples + standards + 1).

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)	Colorimetric Background control (µL)	Fluorometric Background control (µL)
Assay Buffer IV/Pyruvate Assay Buffer	46	47.6	48	49.6
OxiRed Probe/Pyruvate Probe	2	0.4*	2	0.4
Development Enzyme Mix I/Enzyme Mix	2	2	0	0

- Set up plate for standard (50 µL), samples (50 µL) and background control (50 µL).
- Add 50 µL Reaction Mix to each well.
- Incubate plate at RT 30 min protected from light.
- Measure plate at OD570 nm for colorimetric assay or Ex/Em= 535/590 nm for fluorometric assay

Reagent preparation

Briefly centrifuge small vials at low speed prior to opening.

Assay Buffer IV/Pyruvate Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use.

OxiRed Probe/Pyruvate Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. **NOTE: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for few minutes at 37°C.** protected from light and moisture. Once the probe is thawed, use with two months

Development Enzyme Mix I/Pyruvate Enzyme Mix: Reconstitute in 220 µL Assay Buffer IV/Pyruvate Assay Buffer. Aliquot enzyme mix so that you have enough to perform the desired number of assays. Use within two months

Pyruvate Standard: Ready to use as supplied. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays.

Standard preparation

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.

1. Prepare 1 nmol/µL of Pyruvate Standard by adding 5 µL of the Pyruvate Standard to 495 µL of Assay Buffer IV/Pyruvate Assay Buffer.

For the Colorimetric assay:

2. Using 1 nmol/µL Pyruvate standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

For the Fluorometric assay:

2. Prepare 0.1 nmol/µL Pyruvate Standard by diluting 10 µL of 1 nmol/µL Pyruvate Standard into 90 µL of Assay Buffer IV/Pyruvate Assay Buffer.
3. Using 0.1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Conc Pyruvate in well	
				Colorimetric	Fluorometric
1	0	150	50	0 nmol/well	0 nmol/well
2	6	144	50	2 nmol/well	0.2 nmol/well
3	12	138	50	4 nmol/well	0.4 nmol/well
4	18	132	50	6 nmol/well	0.6 nmol/well
5	24	126	50	8 nmol/well	0.8 nmol/well
6	30	120	50	10 nmol/well	1 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).
NOTE: *If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.*

Sample preparation

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, you should complete the Sample Preparation step as well as the deproteinization step before storing the samples. Alternatively, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware that this might affect the stability of your samples and the readings can be lower than expected.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 106 cells).
2. Wash cells with cold PBS.
3. Resuspend cells in 500 µL (4X volumes) Assay Buffer IV/Pyruvate Assay Buffer.
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.
8. Perform deproteinization step as described in the Deproteinization protocol section

NOTE: *If cells are collected by trypsinization, it is essential to neutralize the trypsin with medium. Then all media must be removed and cells washed with PBS. Follow protocol from step 3.*

Tissue samples:

1. Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).
2. Wash tissue in cold PBS.
3. Resuspend tissue in 500 – 1,000 µL (4X volumes) of Assay Buffer IV/Pyruvate Assay Buffer.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
5. Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube.
7. Keep on ice.
8. Perform deproteinization step as described in the Deproteinization protocol section.

Plasma, Serum and Urine and other biological fluids: Can be tested directly by adding sample to the microplate wells. To find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample (1/2 – 1/5 – 1/10).
NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range.*

Deproteinization protocol

NOTE: *Endogenous Lactate Dehydrogenase (LDH) may degrade lactate. Samples containing LDH (such as culture medium or tissue lysate) should be kept at -80°C for storage. Alternatively perform deproteinization as described in this section.*

1. Prepare samples. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.
2. Add PCA to a final concentration of 1M in the homogenate solution and vortex briefly to mix well. NOTE: High protein concentration samples might need more PCA.
3. Incubate samples on ice 5 min. Centrifuge samples at 13000 rpm 2 minutes in a cold centrifuge.
4. Transfer supernatant to a fresh tube.
5. Precipitate excess PCA by adding ice-cold 2 M KOH that equals 34% of the supernatant to your sample (for instance, 34 µL of 2 M KOH to 100 µL sample) and vortexing briefly. This will neutralize the sample and precipitate excess PCA. There may be some gas (CO2) evolution so vent the sample tube.
6. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust the pH with 0.1 M KOH.
7. Centrifuge samples at 13000 rpm 15 minutes in a cold centrifuge.
8. Transfer supernatant to a clean tube, and keep on ice.
9. Samples are now deproteinized, neutralized and PCA has been removed. The samples may now be used directly for the relevant assays.
10. The deproteinized samples will be diluted from the original concentration. To calculate the dilution factor of your final sample, simply apply the following formula:

$$\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{(initial sample volume + vol PCA + vol KOH)}} \text{ X100}$$

Assay Procedure and Detection

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.
- Endogenous compounds may interfere with the reaction. To ensure accurate determination of pyruvate in the test samples, we recommend spiking samples with a known amount of pyruvate standard (6 nmol**).**

Set up Reaction wells:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).
- Background wells = 50 µL Assay Buffer IV/Pyruvate Assay Buffer.
- (Optional Sample Background Control) = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer). Use this for samples with background.

Reaction Mix:

- Prepare 50 µL of Reaction Mix for each reaction:

Component	Colorimetric Reaction Mix (µL)	Fluorometric Reaction Mix (µL)	Colorimetric Background control (µL)	Fluorometric Background control (µL)
Assay Buffer IV/Pyruvate Assay Buffer	46	47.6	48	49.6
OxiRed Probe/Pyruvate Probe	2	0.4*	2	0.4
Development Enzyme Mix I/Enzyme Mix	2	2	0	0

***NOTE:** 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 and increase detection sensitivity.*

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + standards +1).

1. Add 50 µL of the Reaction Mix to standard and samples wells.
2. Add 50 µL of the Background control mix to Sample Background control wells.
3. Mix and incubate at room temperature for 30 minutes protected from light.
4. Measure on a microplate reader.

– Colorimetric assay: measure OD 570nm.
– Fluorometric assay measure Ex/Em = 535/587 nm.

Calculations

– Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
– For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of pyruvate
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Extrapolate sample readings from the standard curve plotted using the following equation:

$$Py = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

6. Concentration of pyruvate in the test samples is calculated as:

$$Pyruvate\ Concentration = \left(\frac{Py}{Sv}\right) * D$$

Where:

Py = Amount of pyruvate (nmol) of sample well (concentration).
Sv = Sample volume (µL) added to the reaction well.
D = Sample dilution factor.

Pyruvate molecular weight: 88.08 g/mol.

7. For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.
8. For spiked samples, the concentration of Pyruvate in sample well is calculated as:

$$Pyruvate = \left(\frac{ODs\ cor}{((ODs + Ts\ cor) - (ODs\ cor))}\right) * Pyr\ Spike\ (pmol)$$

Where:

ODs cor = OD sample corrected.
ODs = OD sample.
Ts = Pyruvate amount from standard curve corrected.

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of inappropriate plate for reader	Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit

	Sample readings above/ below the linear range	Concentrate/ Dilute sample so as to be in the linear range

FAQs

The OD values are very low. What could be the problem?

The most common reason for low OD values is that the Assay buffer was cold, which led to a slow reaction.

Is it possible that the pyruvate in the sample is degraded with time?

Yes, pyruvate is lost fairly rapidly and can get used up by cellular enzymes. It is essential to deproteinize the samples as soon as possible

Should the samples be deproteinized and stored?

Yes, it is recommended that cell/tissue lysates or media is deproteinized and then stored at -80°C if needed.

How much pyruvate is there in serum versus whole blood?

Typically, normal human serum has a pyruvate concentration in the range of 60-150 µM and normal human blood has a pyruvate concentration in the range of 35-100 µM.

What kind of interfering substances can affect the assay results? Undiluted deproteinized samples show values above the expected/reported range.

Compounds such as lactate and a-keto acids in the samples are known to have potential to interfere with the measurement of pyruvate, particularly, 2-Oxobutyrate and oxaloacetate. This could explain why the undiluted deproteinized samples suffer from overestimation.

Technical Support

For all technical or commercial enquiries please go to:

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