ab65331– L- Lactate Assay kit (Colorimetric)

For the rapid, sensitive and accurate measurement of L-Lactate in various samples

View kit datasheet: www.abcam.com/ab65331

(use www.abcam.cn/ab65331 for China, or www.abcam.co.jp/ab65331 for Japan)

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 XII/Lactate Assay Buffer	25 mL	-20°C	-20°C
Enzyme Mix XV/Lactate Enzyme Mix (Lyophilized)	1 vial	-20°C	-20°C
Developer Solution III/Lactate Substrate Mix (Lyophilized)	1 vial	-20°C	4°C
100 mM L(+)-Lactate Standard	100 µL	-20°C	-20°C

Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at OD 450 nm
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom
- Dounce homogenizer (if using tissue)
- Deproteinizing Sample Preparation Kit – TCA (ab204708): for deproteinization step in cell or tissue samples
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as a general use cocktail.

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening

Assay Buffer XII/Lactate Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

Enzyme Mix XV/Lactate Enzyme Mix: Reconstitute with 220 µL of Assay Buffer XII/Lactate Assay Buffer. Pipette up and down to completely dissolve. Keep on ice during the assay. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C.

Developer Solution III/Lactate Substrate Mix: Reconstitute with 220 µL of Assay Buffer XII/Lactate Assay Buffer and mix thoroughly. Keep on ice during the assay. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store aliquots at 4°C.

L(+)-Lactate Standard (100 mM) (MW = 90.08 g/mol): Dilute the Lactate Standard to 1 mM by adding 10 µL of the Lactate Standard to 990 µL of Assay Buffer XII/Lactate Assay Buffer, mix well. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store aliquots at - 20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Discard the working standard dilutions after use as they do not store well.

1. Using 1 mM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (µL)	Assay Buffer XII/Assay Buffer (µL)	Final volume standard in well (µL)	End Conc in well (nmol/well)
1	0	50	50	0
2	2	48	50	2
3	4	46	50	4
4	6	44	50	6
5	8	42	50	8
6	10	40	50	10

Carry out each dilution twice to have enough standard for duplicate readings (2 x 50 µL).

Sample Preparation

General Sample Information

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- Please use fresh samples. If you cannot perform the assay at the same time complete the Sample Preparation step before storing the samples. Alternatively, you can snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you want to test your samples, thaw them on ice. Be aware this might affect the stability of your samples and readings can be lower than expected.
- Add protease inhibitors to sample buffer immediately prior use.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (recommendation = 2 x 10^6 cells).
2. Wash cells with cold PBS.
3. Resuspend the cell pellet in 4x volumes of Assay Buffer XII/Lactate Assay Buffer (~200 µL).
4. Homogenize cells quickly by pipetting up and down a few times.
5. Centrifuge 2 – 5 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.
6. Collect supernatant and transfer to a clean tube and keep on ice.
7. Cell samples may contain endogenous LDH that will degrade lactate. Remove enzyme from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described below.

Tissue samples:

1. Harvest the necessary amount of tissue necessary for each assay (initial recommendation = 10 mg tissue)
2. Wash tissue in cold PBS.
3. Resuspend tissue in 4 – 6X volumes of Assay Buffer XII/Lactate Assay Buffer using a Dounce homogenizer sitting on ice, with 10 – 15 passes.

4. Centrifuge samples for 2 – 5 minutes at top speed at 4°C in a cold microcentrifuge to remove any insoluble material.
5. Collect supernatant and transfer to a clean tube.
6. Keep on ice.
7. Tissue samples may contain endogenous LDH that will degrade lactate. Remove enzyme from sample by using Deproteinizing Sample Preparation Kit – TCA (ab204708). Alternatively, you can perform a PCA/KOH deproteinization step following the protocol described below

Serum samples:

Serum samples can be tested directly; they do not require additional sample preparation.
Recommendation = 0.5- 10 µL serum per well (regular serum contains ~ 0.6 nmol/µL lactate).
We recommend performing several dilutions to ensure readings fall within the standard values.

Alternative deproteinization protocol:

For this step you will need additional reagents:

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

Prepare samples as specified in protocol. You should have a clear protein sample after homogenization and centrifugation. Keep your samples on ice.

1. Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly. NOTE: high protein concentration samples might need more PCA.
2. Incubate on ice for 5 minutes.
3. Centrifuge samples at 13,000 x g for 2 minutes at 4°C in a cold centrifuge and transfer supernatant to a fresh tube. Measure volume of supernatant.
4. Precipitate excess PCA by adding ice-cold 2M 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.
5. After neutralization, it is very important that pH equals 6.5 – 8 (use pH paper to test 1 µL of sample). If necessary, adjust pH with 0.1 M KOH or PCA.
6. Centrifuge at 13,000 x g for 15 minutes at 4°C and collect supernatant.

Samples are now deproteinized, neutralized and PCA has been removed. The samples are now ready to use in the assay.

Sample Recovery

The deproteinized samples will be diluted from the original concentration.
To calculate the dilution factor of your final sample, simply apply the following formula:
% original concentration =

$$\frac{\text{Initial sample volume}}{\text{(Initial sample volume + Vol PCA + Vol KOH)}} \text{ X100}$$

NOTE: *We suggest using different volumes of sample to ensure readings are within the Standard Curve range*.

Assay Procedure

- **We recommend that you assay all standards, controls and samples in duplicate.**
- **Prepare all reagents, working standards, and samples as directed in the previous sections.**
- **Equilibrate all materials and prepared reagents to room temperature prior to use.**

NOTE: *NADH/NADPH present in cell or tissue extracts can generate background in this assay. If you suspect your samples contain NADH/NADPH, set up Sample Background Controls.*

Set up Reaction wells:

Standard wells = 50 µL Standard dilutions.
Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XII/Lactate Assay Buffer).
Sample Background control wells= 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer XII/Assay Buffer).

Reaction Mix:

Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the calculation: **X µL component x (Number reactions** +1).

Components	Reaction Mix (µL)	Background reaction mix (µL)
Assay Buffer XII/Lactate Assay Buffer	46	48
Developer Solution III/Lactate Substrate Mix	2	2
Enzyme Mix XV/Lactate Enzyme Mix	2	0

Add 50 µL of Reaction Mix into each standard and sample well.

1. Add 50 µL of Background Reaction Mix to Sample background control wells.
2. Mix and incubate at room temperature for 30 minutes.
3. Measure output on a microplate reader at OD 450 nm.

The color of the reaction is stable for at least 4 hours.

Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer & reanalyzed. Multiply the concentration by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. If the sample background control is significant, then subtract the sample background control from the sample readings.
3. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
4. Plot the corrected absorbance values for each standard as a function of the final concentration of Lactate.
5. 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).
6. Concentration of L-lactate in the test samples is calculated as:

$$Lactate\ concentration = \left(\frac{La}{Sv}\right) * D$$

Where:

La = amount of Lactic acid in the sample well calculated from standard curve (nmol).
Sv = volume of sample added into the well (µL).
D = sample dilution factor.

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.

- Solubilize Developer Solution III/Lactate Substrate Mix and Enzyme Mix XV/Lactate Enzyme Mix, thaw Lactate Standard and Assay Buffer XII/Lactate Assay Buffer (aliquot if necessary); get equipment ready.
- Prepare Lactate standard dilution [range 2 – 10 nmol/well].
- Prepare samples (including deproteinization step) in optimal dilutions so that they fit standard curve readings.
- Set up plate in duplicate for standard (50µL), samples (50µL), and if appropriate, for sample background control wells (50 µL).
- Prepare a master mix for L-Lactate Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:
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Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer XII/Lactate Assay Buffer	46	48
Developer Solution III/Lactate Substrate Mix	2	2
Enzyme Mix XV/Lactate Enzyme Mix	2	0

- Add 50 µL Reaction Mix to standard and sample wells.
- Add 50 µL Background Reaction Mix to sample background control wells.
- Incubate plate at room temperature for 30 minutes.
- Measure plate at OD 450 nm in a microplate reader.

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 a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use provided protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	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 (< 5 µL) 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 described in the 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 it is within the linear range

FAQs

How many cells should we have in the cell culture to get results that fit in the standard curve?
Typically 1-2 million cells is recommended. This can vary depending on the Lactate content in the cells and hence needs to be optimized.

Is deproteinization necessary for this assay?
Yes, deproteinizing is definitely recommended for metabolically active tissues and cells to ensure lactate in the sample is not used up by enzymes like LDH. Samples can be stored if needed at -80°C after deproteinizing so the assay can be performed at a later stage. For media, it is not as critical but still recommended for best results.

Can medium with phenol red and FBS be used for this assay?
Phenol red will be fine for this assay since small volume of the medium is used per well and after adding Assay Buffer XII/assay buffer to fill up the volume, the color is insignificant.

What components need to be avoided in the medium to assay lactate in cells only?
Ideally, the medium should be devoid of Lactate and pyruvate. Pyruvate can act as a source of lactate for the cells. If cells are grown in medium containing these, simply remove medium, wash with PBS and then lyse the cells to measure intracelllular lactate.

Can this kit be used to measure lactate in bacterial cells/medium?
Although we have not tested this kit with bacteria, since Lactate is the same across kingdoms, this kit should work. Bacterial cells with cell walls might need special lysis reagents

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
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