ab65331
L- Lactate Assay kit
(Colorimetric)

Instructions for use:
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.
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1. BACKGROUND

L – Lactate Assay Kit (Colorimetric) (ab65331) is a kit where lactate is oxidized by lactate dehydrogenase to generate a product which interacts with a probe to produce a color (λmax = 450 nm). The kit detects L(+) Lactate in biological samples such as serum or plasma, cells, culture and fermentation media at a level of 0.02 mM – 10 mM. There is no need for pre-treatment or purification of samples.

Lactate (CH$_3$CH(OH)COO$^-$) plays important roles in many biological processes. Abnormally high concentrations of lactate have been related to disease states such as diabetes and lactic acidosis. L(+) Lactate is the major lactate stereoisomer formed in human intermediary metabolism and is present in blood. D(-)-Lactate can also be found in blood but only at about 1 – 5% of the concentration of L(+) Lactate.

**Principle:**

- **LDH**
- **NADH**
- **NAD**
- **Pyruvate**
- **Lactate**
- **WST**
- **formazan**

Color (λ = 450 nm)
2. ASSAY SUMMARY

Sample preparation*

Standard curve preparation

Add reaction mix and incubate at RT for 30 minutes

Measure optical density (OD450 nm)

*Sample requires deproteinization
3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handle with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. 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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

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

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. MATERIALS SUPPLIED

<table>
<thead>
<tr>
<th>Item</th>
<th>Amount</th>
<th>Storage Condition (Before Preparation)</th>
<th>Storage Condition (After Preparation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Assay Buffer</td>
<td>25 mL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Lactate Enzyme Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Lactate Substrate Mix (Lyophilized)</td>
<td>1 vial</td>
<td>-20°C</td>
<td>4°C</td>
</tr>
<tr>
<td>100 mM L(+)-Lactate Standard</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>
7. 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.
8. TECHNICAL HINTS

- This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.

- Avoid foaming or bubbles when mixing or reconstituting components.

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.

- Ensure plates are properly sealed or covered during incubation steps.

- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.

- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.

- Make sure you have the right type of plate for your detection method of choice.

- Make sure all necessary equipment is switched on and set at the appropriate temperature.
9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening

9.1. Lactate Assay Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2. Lactate Enzyme Mix:

Reconstitute with 220 μL of 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.

9.3. Lactate Substrate Mix:

Reconstitute with 220 μL of 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.

9.4. 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 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.
10. 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.

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

<table>
<thead>
<tr>
<th>Standard #</th>
<th>Volume of Standard (µL)</th>
<th>Assay Buffer (µL)</th>
<th>Final volume standard in well (µL)</th>
<th>End Conc in well (nmol/well)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>48</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>46</td>
<td>50</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>44</td>
<td>50</td>
<td>6</td>
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<tr>
<td>5</td>
<td>8</td>
<td>42</td>
<td>50</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>40</td>
<td>50</td>
<td>10</td>
</tr>
</tbody>
</table>

Carry out each dilution twice to have enough standard for duplicate readings (2 x 50 µL).
11. 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, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Add protease inhibitors to sample buffer immediately prior use.

11.1. Cell (adherent or suspension) samples:

11.1.1. Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

11.1.2. Wash cells with cold PBS.

11.1.3. Resuspend the cell pellet in 4x volumes of Lactate Assay Buffer (~200 µL).

11.1.4. Homogenize cells quickly by pipetting up and down a few times.

11.1.5. Centrifuge 2 – 5 minutes at 4°C at top speed in a cold microcentrifuge to remove any insoluble material.

11.1.6. Collect supernatant and transfer to a clean tube.

11.1.7. Keep on ice.

11.1.8. 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 in section 11.4.
11.2. **Tissue samples:**

11.2.1. Harvest the necessary amount of tissue necessary for each assay (initial recommendation = 10 mg tissue)

11.2.2. Wash tissue in cold PBS.

11.2.3. Resuspend tissue in 4 – 6X volumes of Lactate Assay Buffer using a Dounce homogenizer sitting on ice, with 10 – 15 passes.

11.2.4. Centrifuge samples for 2 – 5 minutes at top speed at 4°C in a cold microcentrifuge to remove any insoluble material.

11.2.5. Collect supernatant and transfer to a clean tube.

11.2.6. Keep on ice.

11.2.7. 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 in section 11.4.

11.3. **Serum samples:**

Serum samples can be tested directly; they do not require additional sample preparation.

Initial recommendation = 0.5- 10 µL serum per well (regular serum contains ~ 0.6 nmol/µL lactate).

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

11.4. **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.
11.4.1. Add ice cold PCA 4 M to a final concentration of 1 M in the homogenate solution and vortex briefly to mix well. **NOTE:** high protein concentration samples might need more PCA.

11.4.2. Incubate on ice for 5 minutes.

11.4.3. 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.

11.4.4. 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.

11.4.5. 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.

11.4.6. 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:

\[
\% \text{ original concentration} = \frac{\text{Initial sample volume}}{\text{Initial sample volume} + \text{Vol PCA} + \text{Vol KOH}} \times 100
\]

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

12. ASSAY PROCEDURE

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- 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.

**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.

12.1. Set up Reaction wells:

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

12.2. Reaction Mix:

12.2.1. 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 following calculation:

\[ X \text{ µL component } \times (\text{Number reactions } + 1) \]

<table>
<thead>
<tr>
<th>Components</th>
<th>Reaction Mix (µL)</th>
<th>Background reaction mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Assay Buffer</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Lactate Substrate Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lactate Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>
12.3. Add 50 µL of Reaction Mix into each standard and sample well.

12.4. Add 50 µL of Background Reaction Mix to Sample background control wells.

12.5. Mix and incubate at room temperature for 30 minutes.

12.6. Measure output on a microplate reader at OD 450 nm.
    The color of the reaction is stable for at least 4 hours.
13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

13.1. Average the duplicate reading for each standard and sample.

13.2. If the sample background control is significant, then subtract the sample background control from the sample readings.

13.3. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.4. Plot the corrected absorbance values for each standard as a function of the final concentration of Lactate.

13.5. 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).

13.6. Concentration of L-lactate in the test samples is calculated as:

\[
\text{Lactate concentration} = \left( \frac{La}{Sv} \right) \times D
\]

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.

Lactic acid molecular weight = 90.08 g/mol.
14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

![Typical Lactate Standard calibration curve using the kit protocol. Background signal subtracted (duplicates; +/- SD).](image1)

**Figure 1**: Typical Lactate Standard calibration curve using the kit protocol. Background signal subtracted (duplicates; +/- SD).

![Relative signal (RFU) in unfiltered human plasma (dilution 1:8), comparing L-lactate signals with background reading (no enzyme) after 10 minutes of incubation (duplicates +/- SD).](image2)

**Figure 2**: Relative signal (RFU) in unfiltered human plasma (dilution 1:8), comparing L-lactate signals with background reading (no enzyme) after 10 minutes of incubation (duplicates +/- SD).
15. 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 Lactate Substrate Mix and Lactate Enzyme Mix, thaw Lactate Standard and 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:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Assay Buffer</td>
<td>46</td>
<td>48</td>
</tr>
<tr>
<td>Lactate Substrate Mix</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Lactate Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

- 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.
### 16. TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Assay not working</strong></td>
<td>Use of ice-cold buffer</td>
<td>Buffers must be at room temperature</td>
</tr>
<tr>
<td></td>
<td>Plate read at incorrect wavelength</td>
<td>Check the wavelength and filter settings of instrument</td>
</tr>
<tr>
<td></td>
<td>Use of a different 96-well plate</td>
<td>Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate</td>
</tr>
<tr>
<td><strong>Sample with erratic readings</strong></td>
<td>Samples not deproteinized (if indicated on protocol)</td>
<td>Use provided protocol for deproteinization</td>
</tr>
<tr>
<td></td>
<td>Cells/tissue samples not homogenized completely</td>
<td>Use Dounce homogenizer, increase number of strokes</td>
</tr>
<tr>
<td></td>
<td>Samples used after multiple free/thaw cycles</td>
<td>Aliquot and freeze samples if needed to use multiple times</td>
</tr>
<tr>
<td></td>
<td>Use of old or inappropriately stored samples</td>
<td>Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use</td>
</tr>
<tr>
<td></td>
<td>Presence of interfering substance in the sample</td>
<td>Check protocol for interfering substances; deproteinize samples</td>
</tr>
<tr>
<td><strong>Lower/Higher readings in samples and Standards</strong></td>
<td>Improperly thawed components</td>
<td>Thaw all components completely and mix gently before use</td>
</tr>
<tr>
<td></td>
<td>Allowing reagents to sit for extended times on ice</td>
<td>Always thaw and prepare fresh reaction mix before use</td>
</tr>
<tr>
<td></td>
<td>Incorrect incubation times or temperatures</td>
<td>Verify correct incubation times and temperatures in protocol</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Standard readings do not follow a linear</td>
<td>Pipetting errors in standard or reaction</td>
<td>Avoid pipetting small volumes (&lt; 5 µL) and prepare a master mix whenever possible</td>
</tr>
<tr>
<td>pattern</td>
<td>mix</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Air bubbles formed in well</td>
<td>Pipette gently against the wall of the tubes</td>
</tr>
<tr>
<td></td>
<td>Standard stock is at incorrect concentration</td>
<td>Always refer to dilutions described in the protocol</td>
</tr>
<tr>
<td>Unanticipated results</td>
<td>Measured at incorrect wavelength</td>
<td>Check equipment and filter setting</td>
</tr>
<tr>
<td></td>
<td>Samples contain interfering substances</td>
<td>Troubleshoot if it interferes with the kit</td>
</tr>
<tr>
<td></td>
<td>Sample readings above/ below the linear</td>
<td>Concentrate/ Dilute sample so it is within the linear range</td>
</tr>
</tbody>
</table>
17. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- EDTA > 1mM

18. 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 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 intracellular 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.
19. NOTES
UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

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