ab65330

L-Lactate Assay Kit
(Colorimetric/Fluorometric)

Instructions for Use

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

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/Fluorometric) (ab65330), lactate specifically reacts with an enzyme mix to generate a product, which interacts with lactate probe to produce color (570 nm) and fluorescence (Ex/Em = 535/587 nm).

This assay provides a convenient means for detecting L(+)-Lactate in biological samples such as in blood circulation, in cells, in culture mediums, in fermentation mediums, etc. There is no need for pre-treatment or purification of samples. This assay can detect 0.001-10 mM of various Lactate samples.

Abnormally high concentrations of lactate have been related to disease states such as diabetes and lactate acidosis. L(+)-Lactate is the major stereo-isomer of lactate formed in human intermediary metabolism and is present in blood. D(-)-Lactate is also present but only at about 1-5% of the concentration of L(+)-Lactate.
2. **ASSAY SUMMARY**

- Sample preparation

  ↓

- Standard curve preparation

  ↓

- Add reaction mix and incubate 30 min at RT

  ↓

- Measure optical density (OD570 nm) or fluorescence (Ex/Em = 535/587 nm)
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. Modifications to the kit components or procedures may result in loss of performance.

4. **STORAGE AND STABILITY**

Store kit at -20ºC 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 section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**
5. **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 Probe</td>
<td>200 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>Lactate Enzyme Mix</td>
<td>1 vial</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
<tr>
<td>100 nmol/µL L(+)-Lactate Standard</td>
<td>100 µL</td>
<td>-20°C</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

6. **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 OD570 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 tissue)
- PBS

If performing deproteinization step, additional reagents are required:

- Perchloric acid (PCA) 4M, ice cold
- Potassium Hydroxide (KOH) 2M
- 10 kD Spin Columns (ab93349) – for fluid samples, if not performing PCA precipitation
7. **LIMITATIONS**

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.
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.

- Keep enzymes and heat labile components and samples on ice during the assay.

- Make sure all buffers and developing solutions are at room temperature before starting the experiment.

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

- Avoid foaming or bubbles when mixing or reconstituting components.

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.

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

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

- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
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 protected from light and moisture.

9.2 **Lactate 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.* Aliquot probe so that you have enough to perform the desired number of assays. Store at -20°C, protect from light and moisture. Once probe is thawed, use within two months.

9.3 **Lactate Enzyme Mix:**

Dissolve in 220 μL Lactate Assay Buffer. Pipette up and down to completely dissolve. Aliquot enzyme mix so that you have enough to perform the desired number of assays. Store at -20°C. Use within two months.

9.4 **Lactate 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. Store at -20°C.
10. **STANDARD PREPARATION**

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

10.1  **For the colorimetric assay:**

10.1.1 Prepare 0.5 mL of 1 nmol/µL Lactate standard by adding 5 µL of the 100 nmol/µL Lactate Standard to 495 µL of Lactate Assay Buffer.

10.1.2 Using 1 nmol/µL Lactate 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 [L-Lactate] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 nmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>2 nmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>4 nmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>6 nmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>8 nmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>10 nmol/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 µL).
10.2 **For the flurometric assay:**

10.2.1 Prepare 500 µL of 1 nmol/µL Lactate standard by adding 5 µL of the Lactate Standard to 495 µL of Lactate Assay Buffer.

10.2.2 Prepare 1 mL of 0.01 nmol/µL Lactate Standard by diluting 10 µL of 1 nmol/µL standard to 990 µL of Lactate Assay Buffer.

10.2.3 Using 0.01 nmol/µL 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 [L-Lactate] in well</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>150</td>
<td>50</td>
<td>0 pmol/well</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>144</td>
<td>50</td>
<td>20 pmol/well</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>138</td>
<td>50</td>
<td>40 pmol/well</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>132</td>
<td>50</td>
<td>60 pmol/well</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>126</td>
<td>50</td>
<td>80 pmol/well</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>120</td>
<td>50</td>
<td>100 pmol/well</td>
</tr>
</tbody>
</table>

Each dilution has enough amount of standard to set up duplicate readings (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.
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 as well as the deproteinization step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze samples 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 however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Serum, plasma, cell culture medium and other liquid samples:

**Serum/Plasma:** Recommended dilutions = 10 – 40X (colorimetric) / 400 – 8000X (fluorometric).

**Cell supernatants:** Recommended dilutions = 40 – 160X (colorimetric) / 10,000 – 30,000X (fluorometric).

Serum samples and culture medium (as it contains FBS) generally contain high amount of proteins, so they should be deproteinized with a 10kD Spin column (ab93349).

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

Endogenous compounds in the sample may interfere with the reaction. To ensure accurate determination of L-Lactate in the test samples, we recommend spiking the samples with a known amount of Standard (4 nmol)
12. **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.

12.1 **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 sample control wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer).

12.2 **Reaction Mix (COLORIMETRIC ASSAY):**
Prepare 50 µL Reaction Mix for each reaction:

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

12.3 **Reaction Mix (FLUOROMETRIC ASSAY):**
Prepare 50 µL Reaction Mix for each reaction:

<table>
<thead>
<tr>
<th>Component</th>
<th>Reaction Mix (µL)</th>
<th>Background Control Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Assay Buffer</td>
<td>47.6</td>
<td>49.6</td>
</tr>
<tr>
<td>Probe</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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:

\[ X \, \mu\text{L component} \times (\text{Number samples} + \text{standards} + 1) \]

12.4 **Add 50 µL of Reaction Mix into each standard and sample well.**
12.5 Add 50 µL of Background Control Mix to background wells.

12.6 Mix and incubate at room temperature for 30 minutes protected from light.

12.7 Measure output on microplate reader.
- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.
13. **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).

13.1 Average the duplicate reading for each standard and sample.

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

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

13.4 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).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

\[
L_a = \left( \frac{\text{Corrected absorbance/RFU} - (y – \text{intercept})}{\text{Slope}} \right)
\]

13.6 Concentration of samples in the test samples is calculated as:

\[
\text{Lactate Concentration} = \left( \frac{L_a}{S_v} \right) \times D
\]

Where:
- \(L_a\) = Lactate amount from standard curve (nmol).
- \(S_v\) = Sample volume added in sample wells (µL).
- \(D\) = Sample dilution factor.
- Lactate acid molecular weight: 90.08 g/mol.
13.7 For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

13.8 For spiked samples, the concentration of L-Lactate in sample well is calculated as:

\[
[L\text{Lactate}] = \left( \frac{ODs\text{ cor}}{ODs + Ts\text{ cor}} \right) \times Lactate\text{ Spike (nmol)} - (ODs\text{ cor})
\]

Where:

ODs cor = OD sample corrected

ODs = OD samples

Ts cor = Lactate amount from standard curve corrected
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 colorimetric reading.](image)

**Figure 1**: Typical lactate standard calibration curve using colorimetric reading.
Figure 2: Typical lactate standard calibration curve using fluorometric reading.

\[ y = 273.94x + 4168.3 \]

Figure 3: Quantitation of lactate in Human saliva and serum.
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 Enzyme and mix. Thaw Lactate Standard, Lactate Probe and Lactate Assay Buffer ( 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).
- Set up plate for standard (50µL) and samples (50µL) and background wells (50µL).
- Prepare Lactate Reaction Mix (Number samples + standards + 1).
- Prepare Background Control Reaction Mix (Number of background samples + 1).

<table>
<thead>
<tr>
<th>Component</th>
<th>Colorimetric/ Background Reaction Mix (µL)</th>
<th>Fluorometric/ Background Reaction Mix (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate Assay Buffer</td>
<td>46 / 48</td>
<td>47.6 / 49.6</td>
</tr>
<tr>
<td>Probe</td>
<td>2 / 2</td>
<td>0.4 / 0.4</td>
</tr>
<tr>
<td>Enzyme Mix</td>
<td>2 / 0</td>
<td>2 / 0</td>
</tr>
</tbody>
</table>

- Add 50 µL Lactate Reaction Mix to standard and samples.
- Add 50 µL Background Control Reaction Mix to background wells.
- Mix and incubate plate at RT for 30 mins.
- Measure plate at OD 570 nm for colorimetric or Ex/Em= 535/587 nm for fluorometric assay.
# Troubleshooting

## Problem 1: Assay not working
- **Use of ice-cold buffer**
  - **Cause**: Buffers must be at room temperature
- **Plate read at incorrect wavelength**
  - **Solution**: Check the wavelength and filter settings of instrument
- **Use of inappropriate plate for reader**
  - **Solution**: Colorimetry: Clear plates, Fluorescence: Black plates (clear bottom)

## Problem 2: Sample with erratic readings
- **Samples not deproteinized (if indicated on protocol)**
  - **Solution**: Use PCA precipitation protocol for deproteinization
- **Cells/tissue samples not homogenized completely**
  - **Solution**: Use Dounce homogenizer (increase number of strokes); observe for lysis under microscope
- **Samples used after multiple free/thaw cycles**
  - **Solution**: Aliquot and freeze samples if needed to use multiple times
- **Use of old or inappropriately stored samples**
  - **Solution**: Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
- **Presence of interfering substance in the sample**
  - **Solution**: Check protocol for interfering substances; deproteinize samples

## Problem 3: Lower/higher readings in samples and standards
- **Improperly thawed components**
  - **Solution**: Thaw all components completely and mix gently before use
- **Allowing reagents to sit for extended times on ice**
  - **Solution**: Always thaw and prepare fresh reaction mix before use
- **Incorrect incubation times or temperatures**
  - **Solution**: Verify correct incubation times and temperatures in protocol
<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard readings do not follow a linear pattern</td>
<td>Pipetting errors in standard or reaction mix</td>
<td>Avoid pipetting small volumes and prepare a master mix whenever possible</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 on 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 range</td>
<td>Concentrate/ Dilute sample so as to be in the linear range</td>
</tr>
</tbody>
</table>
17. FAQs

Is there difference between incubation of 37°C and room temperature?
The difference between room temperature and 37°C is not significant enough to affect the results. The reaction is slower at RT.

What is the explanation for seeing lower readings at higher conc. of sample?
Higher concentration of lactate does have an inhibitory effect that leads to lower readings. So, smaller amount of sample is recommended to generate values that fit in the linear part of the standard curve. When there is lactate overdose, the reaction will appear dark pink and then turns brown.

What kind of medium should be used with this assay?
Medium devoid of Lactate and/or pyruvate should be used. Medium containing FBS should be deproteinized to remove LDH which can degrade Lactate.

Will the phenol red in the media affect the assay readout?
Very low amounts of media are used for each sample. This will generate a very low background at the worst. Please use only media as a background control and subtract this reading from all sample readings to accommodate for the phenol red.

Is it essential to deproteinize samples for this assay?
Yes, it is highly recommended to deproteinize samples/medium to remove enzymes such as LDH which can quickly degrade lactate.

Can frozen samples be used with this assay?
Fresh samples are always preferred over frozen samples. However, frozen samples (-80°C) can also be used, provided, they were frozen
right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods. For cell lysates/tissue homogenates or cell culture media, storing after deproteinizing is recommended.

**Does this assay detect D-lactate as well?**

No, this assay is specific for L-lactate. If there is a substantial amount of D-lactate in the sample, a parallel background control can be run to exclude any possibility of interference.
18. **INTERFERENCES**

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

- Lactate dehydrogenase (LDH) (present in serum) will degrade lactate.