ab65330 L-Lactate Assay Kit (Colorimetric/Fluorometric)

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

For overview, typical data and additional information please visit: www.abcam.com/ab65330
(use abcam.cn/ab65330 for China, or abcam.co.jp/ab65330 for Japan)

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.

Materials Supplied and Storage

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

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer II/Lactate Assay Buffer	25 mL	-20°C	-20°C
OxiRed Probe/Lactate Probe	200 µL	-20°C	-20°C
Lactate Enzyme Mix	1 vial	-20°C	-20°C
100 nmol/µL 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 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)
- 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

Reagent Preparation

Assay Buffer II/Lactate Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C protected from light and moisture.

OxiRed Probe/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 OxiRed Probe/probe so that you have enough to perform the desired number of assays. Store

at -20°C, protect from light and moisture. Once OxiRed Probe/probe is thawed, use within two months.

Lactate Enzyme Mix: Dissolve in 220 µL Assay Buffer II/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.

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.

Standard Preparation

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

Prepare 500 µL of 1 nmol/µL Lactate standard by adding 5 µL of the 100 nmol/ µL Lactate Standard to 495 µL of Assay Buffer II/Lactate Assay Buffer.

For the colorimetric assay: Using 1 nmol/µL Lactate Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.

For the flurometric assay: Prepare 1 mL of 0.01 nmol/µL Lactate Standard by diluting 10 µL of 1 nmol/µL standard to 990 µL of Assay Buffer II/Lactate Assay Buffer. Using 0.01 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 II/Assay Buffer (µL)	Final volume standard in well (µL)	End [L-Lactate] in well (Colorimetric)	End [L-Lactate] in well (Fluorometric)
1	0	150	50	0 nmol/well	0 nmol/well
2	6	144	50	2 nmol/well	0.02 nmol/well
3	12	138	50	4 nmol/well	0.04 nmol/well
4	18	132	50	6 nmol/well	0.06 nmol/well
5	24	126	50	8 nmol/well	0.08 nmol/well
6	30	120	50	10 nmol/well	0.1 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

- 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.

Cell (adherent or suspension) samples:

1. Harvest the amount of cells necessary for each assay (initial recommendation = 2×10^6 cells).
2. Wash cells with cold PBS.
3. Resuspend the cell pellet in 4x volumes of Assay Buffer II/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.
7. Keep on ice.
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 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 II/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, plasma and other liquid samples:

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

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

Assay Procedure

• Equilibrate all materials and prepared reagents to room temperature prior to use.
• It is recommended to assay all standards, controls and samples in duplicate.

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 II/Assay Buffer).
- Background sample control wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer II/Assay Buffer).
2. Prepare 50 µL Reaction Mix for each reaction. 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)

Component	COLORIMETRIC ASSAY		FLUOROMETRIC ASSAY	
	Reaction Mix (µL)	Background Control Mix (µL)	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer II/Lactate Assay Buffer	46	48	47.6	49.6
OxiRed Probe/Probe	2	2	0.4	0.4
Enzyme Mix	2	0	2	0

3. Add 50 µL of Reaction Mix into each standard and sample well.
4. Add 50 µL of Background Control Mix to background wells.
5. Mix and incubate at room temperature for 30 minutes protected from light.
6. Measure output on microplate reader.
- Colorimetric assay: measure OD570 nm.
- Fluorometric assay: measure Ex/Em = 535/587 nm.

Calculation:

Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the sample background control reading from sample reading. Plot the Lactate Standard Curve. For unspiked samples, apply the corrected OD to the Lactate Standard Curve to get B nmol of Lactate in the sample well.

Sample Lactate concentration (C) = B/V X D nmol/µl or mM

Where:

$\quad$ **B** is the amount of Lactate in the sample well (nmol)

$\quad$ **V** is the sample volume added into the reaction well (µl)

$\quad$ **D** is the sample dilution factor

Note: For spiked samples, correct for any sample interference by subtracting the sample reading from spiked sample reading.

$For\ spiked\ samples, Lactate\ amount\ in\ sample\ well\ (B)\ =$

$$\left(\frac{(OD_{sample(corrected)})}{(OD_{sample+Lactate\ Std(corrected)})\text{-}(OD_{sample(corrected)})} \right) \times Lactate\ spike\ (nmol)$$

Technical Support

For all technical or commercial enquiries please go to:
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