



**KB03041**

# **Lactate Assay Kit**

**96 well plate  
100/200/400 tests**

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## 1. General information

### PRECAUTIONS

Please read this manual carefully before beginning the assay.

This product is designed for **research use only**. It is not approved for human or animal use or clinical diagnosis. All chemicals should be handled with care and in accordance with laboratory safety practices. It is recommended to use basic Personal Protective Equipment.

Do not use after the expiration date stated on the packaging.

Do not mix or substitute reagents or materials from other kit batches or vendors.

For the **material safety data sheet** (MSDS) please contact us at [info@bioquochem.com](mailto:info@bioquochem.com)

### TECHNICAL RECOMMENDATIONS

Store reagents as indicated in **Materials and storage** section.

Be sure to keep the bottle capped when not in use.

Let the components reach room temperature (RT) before use.

Immediately before use, gently invert and rotate reagent bottles several times to mix the contents thoroughly.

Avoid foaming or bubbles when mixing or reconstituting components.

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

Be sure to use the optimal microplate for the assay. Flat bottom transparent microplates for UV/VIS applications, and black microplates for fluorescence measurements.

## 2. Technical specifications

### Available sizes

100/200/400 tests

### Required sample volume

50 µL/test

### Compatible samples

Animal tissue homogenates, cells, plasma, serum, and other biological samples

### Type of detection

Colorimetric (450 nm)

### 3. Materials and storage

#### MATERIALS SUPPLIED

Store kit components as indicated below:

Item	No. Tests	Units	Storage
Reagent A	100	1	4 °C
	200	2	
	400	4	
Reagent B	100	1	-20 °C
	200	2	
	400	4	
Reagent C	100	1	-20 °C
	200	2	
	400	4	
Reagent D	100	1	-20 °C
	200	2	
	400	4	
Reagent E	100	1	-20 °C
	200	2	
	400	4	
Standard	100	1	-20 °C
	200	1	
	400	1	
Transparent 96-Well Microplate	100	1	RT
	200	2	
	400	4	

#### MATERIALS NEEDED BUT NOT SUPPLIED

- Double distilled water (ddH<sub>2</sub>O) as Milli-Q Ultrapure Water
- Incubator
- Labware materials (micropipettes, tubes, stirring/mixing equipment)
- Colorimetric microplate reader – equipped with filter for OD 450 nm

#### STORAGE CONDITIONS

On receipt, store kit components as indicated above. Under these conditions, the reagents are stable in the original packaging until the expiration date indicated on the outside of the box. **Reagents D and E provided are light sensitive** and should be stored in the dark. Standard solutions are unstable in the presence of oxygen. Prepare a fresh set of standards for every use.

## 4. Introduction

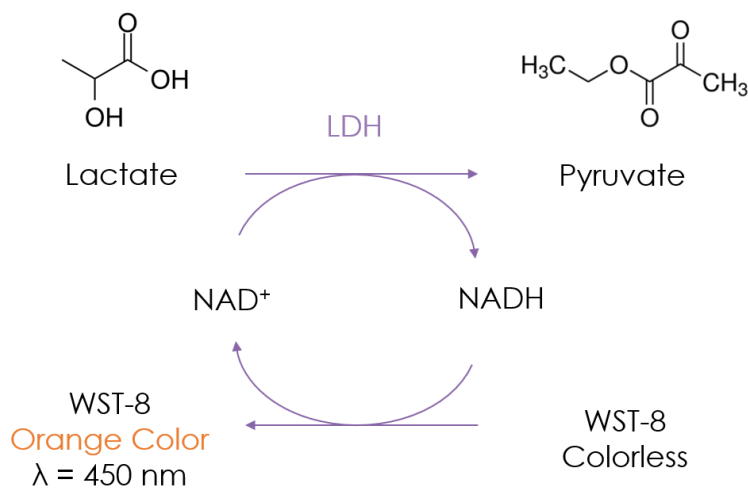
Lactate is a metabolite formed in animals from pyruvate by the action of the lactate dehydrogenase (LDH). It is formed during anaerobic conditions such as exercise. Its accumulation occurs when the energy demand in tissues exceeds the availability of oxygen in the blood.

L(+)-Lactate is the major lactate stereoisomer formed in human intermediary metabolism. Abnormally high concentrations of lactate have been related to pathological conditions such as cancer, diabetes and lactate acidosis.

**BQC Lactate Assay Kit is a quick, easy, and reproducible assay to quantify lactate in a wide variety of samples.**

## 5. Assay Principle

In this assay, lactate is oxidized to pyruvate while  $\text{NAD}^+$  is reduced to NADH by LDH. NADH interacts with a probe yielding a final colorimetric product. The amount of this product, that can be measured at 450 nm, is proportional to the amount of lactate present in the sample.



*Lactate Assay Principle*

## 6. Assay preparation

### REAGENT PREPARATION

All assay reagents not listed below are ready to use as supplied. Allow the reagents to reach room temperature before use and briefly centrifuge small vials at low speed before opening.

❗ **CAUTION:** Working Solutions must be freshly prepared and used immediately. Keep in the dark.

**Lactate Working Solution:** For 100 tests, prepare 5.5 mL of lactate working solution by mixing 3.1 mL of **Reagent A**, 1 mL of **Reagent B**, 0.8 mL of **Reagent C**, 0.5 mL of **Reagent D** and 0.1 mL of **Reagent E**.

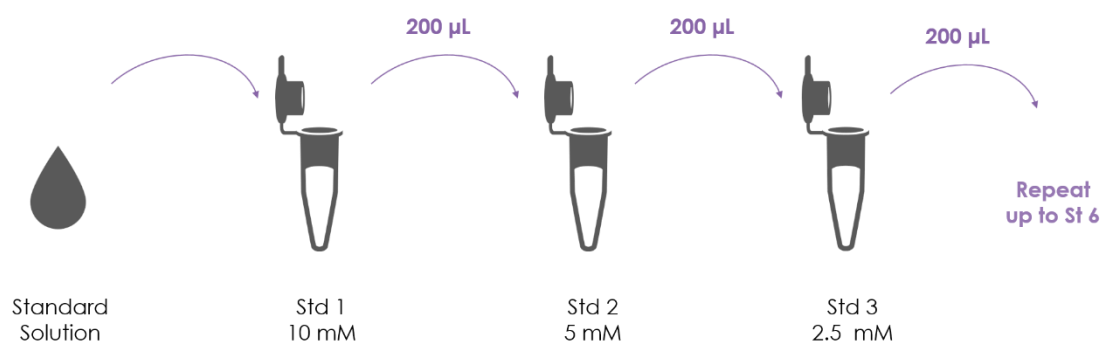
❗ **Optional:** if sample blanks are assayed, prepare half of the **Lactate Working Solution** (2.75 mL) and 2.75 mL of **Background Working Solution** by mixing 2.05 mL of **Reagent A**, 0.4 mL of **Reagent C**, 0.25 mL of **Reagent D** and 0.05 mL of **Reagent E**.

#### Standard Solution (L(+)-Lactate Standard):

- Mix 20  $\mu\text{L}$  of **Standard** with 980  $\mu\text{L}$  of **Reagent A** to obtain a 2 mM solution.
- Dilute the mixture 1:4 with **Reagent A** (i.e., by adding 250  $\mu\text{L}$  of previous solution into 750  $\mu\text{L}$  of Reagent A) and mix well. Use this **Standard Solution 0.5 mM** to prepare the standard calibration curve.

## STANDARD CALIBRATION

Prepare Lactate standards for the calibration curve from the Standard Solution by making serial dilutions according to the following figure and table. Prepare the standards immediately prior to each assay. Vortex tubes thoroughly. Discard standard solutions after use.



*Preparation of standards from the Standard Solution making serial dilutions*

Standard	Standard Solution (µL)	Reagent A (µL)	Standard Concentration (mM)
Std 1	200 Standard Solution 0.5 mM	0	0.5
Std 2	200 Std1	200	0.25
Std 3	200 Std2	200	0.125
Std 4	200 Std3	200	0.0625
Std 5	200 Std4	200	0.0313
Std 6 (Reagent Blank)	0	200	0



## PLATE SET UP

**BQC recommends running the standards and samples at least in duplicate (triplicate recommended).** There is no specific pattern for using the wells on the plate. A proposed layout of standards (Std) and samples (S) to be measured in duplicate is shown below.

**NOTE:** If sample blanks are included in the assay, it is necessary to reserve some wells of the plate for these blanks

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 1	Std 1	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
B	Std 2	Std 2	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
C	Std 3	Std 3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
D	Std 4	Std 4	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
E	Std 5	Std 5	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
F	Std 6	Std 6	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
G	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
H	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42

*Example of plate layout for the Lactate Assay Kit*

## 7. Sample preparation

The following sample preparation protocols are intended as a guide only. The optimal conditions for sample preparation must be determined by the end user. It is recommended to use fresh samples. If it is not possible, aliquot and store samples with minimal freeze/thawing.

**BQC Lactate Assay Kit can be used to determine lactate content in a wide variety of samples like animal tissues, cells, plasma, serum and other biological fluids.**

**Animal Tissues.** Weigh 0.1 g tissue. Add 1 mL of cold buffer and homogenize on ice. Centrifuge at 12000 g for 5 minutes at 4 °C. Use the supernatant and keep on ice to be tested.

**Cells and bacteria.** Collect  $5 \cdot 10^6$  cells into the centrifuge tube, wash cells or bacteria with cold PBS and discard supernatant after centrifugation. Add 1 mL of buffer to ultrasonically disrupt the cells or bacteria for 5 minutes. Centrifuge at 12000 g for 5 minutes at 4 °C. Use supernatant for the assay and place it on ice to be tested.

**Serum, plasma, and other biological fluids** can be tested directly.

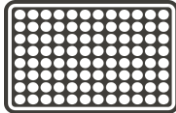






❗ **NOTE:** Endogenous LDH may degrade lactate. Samples containing LDH (culture medium or tissues) should be filter through a 10kDa MW spin filter to remove all proteins and then kept at -80 °C for storage.

Reagents and materials required for sample preparation are not supplied with the kit. Before doing sample preparation, consider the volume of sample required per test; see **Technical specifications** section.

Make sure that interfering substances present in the sample do not give a significant background. Run proper blanks as necessary. It is recommended to assay different sample dilutions to ensure the values fall within the standard curve.

## 8. Assay protocol

Prepare and mix all reagents thoroughly before use. Each standard, sample or blank should be assayed at least in duplicate.

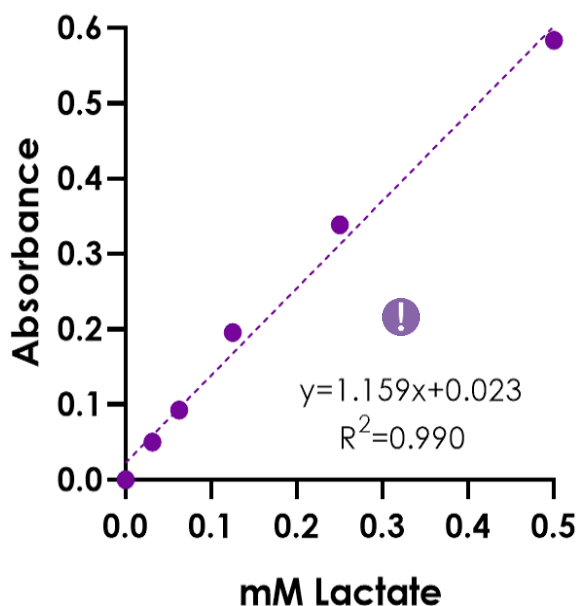
- 1  Set up the plate design
- 2  **Standard** and **sample** wells: add **50 µL** of sample or standard to each well  
  
 **Optional:** Sample blank wells: Add **50 µL** of **Sample**
- 3  Add **50 µL** of **Lactate Working Solution** to each well  
  
 **Optional:** Sample blank wells: Add **50 µL** of **Background Working Solution**
- 4  **Incubate** the reaction for **30 minutes at 37 °C** in the **dark**
- 5  Read the **absorbance** of all wells at **450 nm**

If you need to **adapt this kit** for another form of the assay (for example cuvette), **contact us at** [info@bioquochem.com](mailto:info@bioquochem.com)

## 9. Data analysis

### ANALYSIS OF THE STANDARDS

- Calculate the average absorbance of the standards.
- Subtract the average absorbance of the reagent blank (**Std 6**) from the average absorbance of all the standards to obtain the blank-corrected absorbance of the standards.
- Create a standard curve by plotting the blank-corrected absorbance of the standards as a function of the standard concentration (see **STANDARD CALIBRATION** section). A typical standard curve ( $y = \text{slope} \cdot x \pm \text{intercept}$ ) for this assay is shown below.



*Standard curve for Lactate Assay Kit*

- ! This standard curve is an example of the data typically obtained with this kit. **DO NOT USE** this standard curve to calculate the lactate content of your samples. A new standard curve must be performed by the end user.

## ANALYSIS OF THE SAMPLES

- Calculate the average absorbance of the samples.
- Subtract the average absorbance of the reagent blank (**Std 6**) from the average absorbance of each sample to obtain the blank-corrected absorbance of the samples (**A<sub>s</sub>**).
  - ❗ If sample blanks are assayed and they are significant, subtract them from the average absorbance of the samples
- Calculate the lactate content from a sample as mM lactate using the equation obtained from the linear regression of the standard curve by substituting blank-corrected absorbance for each sample (**A<sub>s</sub>**).

$$\text{Lactate (mM)} = \left( \frac{A_s - \text{intercept}}{\text{slope}} \right)$$

When working with diluted samples the concentration values obtained must be multiplied by the dilution factor to obtain the lactate (mM) value of the undiluted sample.

## 10. Troubleshooting

This troubleshooting table provides potential sources and solutions for common problems observed with BQC Assay Kits. **The problems listed below could occur when using any BQC Assay Kit.** They are not specific for this Assay Kit.

Problem	Possible Cause	Recommended Solution
Wells have color but there is no reading	Plate read at incorrect wavelength	Check the wavelength used in the assay
	Incorrect microplate	Use the correct microplate for your application UV/Vis: transparent Fluorescence: black wells/transparent bottom
Standard readings do not follow a linear pattern	Pipetting errors in preparation of standards	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique
	Standard stock is at incorrect concentration	Always refer to dilutions described in <b>Assay preparation</b>
	Improperly thawed reagents	Thaw all components completely and mix well before use
	Use of improperly stored reagents	Store the components appropriately Use fresh components from the standard curve
	Incorrect incubation times or temperatures	Refer to <b>Refer to Assay protocol</b>
Dispersion of standard and sample readings	Pipetting errors	Avoid pipetting small volumes (<5 µL) Be careful not to splash from well to well
	Air bubbles formed in well(s)	Use reverse pipetting technique

Problem	Possible Cause	Recommended Solution
Sample erratic values	Samples contain interfering substances	Dilute sample further (if possible)
	Inappropriately stored samples or samples used after multiple freeze-thaw cycles	Use fresh samples or store appropriately until use
	Samples not deproteinized	Use an appropriate deproteinization protocol
	Cells/Tissue samples not homogenized completely	Repeat the sample homogenization
	Inappropriate sample dilution buffer	Refer to <b>Assay preparation</b>
Sample reading fall outside the detection range	Samples are too diluted/concentrated No analyte/activity is observed in the sample	Re-assay using different sample dilutions

## STILL HAVING PROBLEMS?

Contact BQC if you have any further questions, our team will be pleased to help you:



Phone

+ 34 985 26 92 92



E-mail

[info@bioquochem.com](mailto:info@bioquochem.com)



Business hours

Monday-Thursday: 8.30 to 17.00 (CEST)  
Friday: 8.00 to 15.00 (CEST)

## 11. Additional information

**Lactate Assay Kit** is a quick (< 45 minutes) and precise (RSD < 5%) assay for determining lactate content in a wide variety of samples.

NADH and NADPH from cells and tissues have been reported to interfere with this assay and, therefore, sample blanks are recommended. Endogenous LDH can degrade lactate, follow **Sample Preparation** recommendations.

If unexpected results are obtained running your samples, please contact us at [info@bioquochem.com](mailto:info@bioquochem.com)

## 12. Related products

More products available at [bioquochem.com](http://bioquochem.com)

Reference	Product
KB03042	Lactate Dehydrogenase (LDH) Assay Kit
KB03051	Uric Acid Assay Kit
KB03039	Cys Assay Kit



## 13. Warranties and limitation of liability

BQC shall not in any event be liable for incidental, consequential or special damages of any kind resulting from any use or failure of the products, even if BQC has been advised of the possibility of such damage including, without limitation, liability for loss of use, loss of work in progress, downtime, loss of revenue or profits, failure to realize savings, loss of products of buyer or other use or any liability of buyer to a third party on account of such loss, or for any labor or any other expense, damage or loss occasioned by such product including personal injury or property damage is caused by BQC's gross negligence. Any and all liability of BQC hereunder shall be limited to the amounts paid by the buyer for the product.

Buyer's exclusive remedy and BQC's sole liability hereunder shall be limited to a refund of the purchase price, or the replacement of all material that does not meet our specifications.

Said refund or replacement is conditioned on buyer giving written notice to BQC within 30 days of shipment.

**Expiration date:** 1 year from the date of fabrication. Expiration date is indicated on the outside of the box.

For further details, please refer to our website [bioquochem.com](https://bioquochem.com)



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