Datasheet for ABIN5067557

Lactate Assay Kit (Colorimetric)

3 Images

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Overview

Quantity:	100 tests
Reactivity:	Bacteria
Application:	Biochemical Assay (BCA)

Product Details

Purpose:	Lactate Assay Kit measures lactate within biological samples.
Sample Type:	Urine, Plasma, Serum, Saliva
Detection Method:	Colorimetric
Sensitivity:	1.5 µM
Characteristics:	Lactate Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of total lactate present in biological samples in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, lactate standards and unknown samples. Sample lactate concentrations are determined by comparison with a known lactate standard. The kit has a detection sensitivity limit of 1.5 µM L-Lactate.
Components:	1. Lactate Standard : One 100 µL tube at 100 mM 2. 10X Assay Buffer : One 25 mL bottle 3. Colorimetric Probe : One 50 µL amber tube 4. HRP : One 100 µL tube at 100 U/mL in glycerol Box 2 (shipped on blue ice packs)

Target Details

Background:	Lactic Acid is an alpha hydroxyl acid that can ionize a carboxyl proton to yield the lactate ion,

Target Details

the latter of which exists as two optical isomers L-Lactate and D-Lactate. The enzyme lactate dehydrogenase catalyzes the conversion of pyruvate to lactate in animals during the process of fermentation. Depending on the levels of exercise, blood levels of lactate can vary between 1 and 20 mM. In medicine, lactate is a component of intravenous fluids such as Hartmann's solution. These fluids are often used when blood loss occurs due to surgery or injury. In the brain, lactate, like glucose, is thought to be one of the main sources of energy. High levels of lactate have been found in the extracellular fluid surrounding neurons due to the high metabolic activity of glial cells. In the food industry, lactic acid is found in cheeses, milk, and various breads. In winemaking, lactic acid bacteria are used to reduce malic acid levels and therefore decrease the sharpness in flavor. Finally, in the detergent industry lactic acid has been used as an anti-bacterial agent as well as a soap-scum removal agent and descaler.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Detects L-lactate in plasma, serum, urine, saliva, and lysate samples • Detection sensitivity of approximately 1.5uM L-lactate • Lactate standard curve included
Protocol:	Lactate is oxidized by lactate oxidase into pyruvate and hydrogen peroxide. The hydrogen peroxide is then detected with a highly specific colorimetric probe. Horseradish peroxidase catalyzes the reaction between the probe and hydrogen peroxide, which bind in a 1:1 ratio. Samples and standards are incubated for 30-45 minutes and then read with a standard 96-well colorimetric plate reader . Samples are compared to a known concentration of lactate standard within the 96-well microtiter plate format. . Lactate assay principle.
Reagent Preparation:	• 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity. • Reaction Mix: Prepare a Reaction Mix by diluting the Colorimetric Probe 1:100, HRP 1:500, and Lactate Oxidase 1:200 in 1X Assay Buffer. For example, add 10 µL Colorimetric Probe stock solution, 2 µL HRP stock solution, and 5 µL of Lactate Oxidase to 983 µL of 1X Assay Buffer for a total of 1 mL. This Reaction Mix volume is enough for 20 assays. The Reaction Mix is stable for 1 day at 4 °C. Note: Prepare only enough for immediate use by scaling the above example proportionally.
Sample Preparation:	• Cell culture supernatants: Cell culture media containing lactate should be avoided. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The cell conditioned media may be assayed directly or diluted as necessary. Prepare the Lactate standard curve in non-conditioned media without lactate. Note: Maintain pH between 7 and 8 for optimal working

Application Details

	conditions as the Colorimetric Probe is unstable at high pH (>8.5). • Tissue lysates: Sonicate or homogenize tissue sample in cold PBS or 1X Assay Buffer and centrifuge at 10000 x g for 10 minutes at 4 °C. Perform dilutions in 1X Assay Buffer. • Cell lysates: Resuspend cells at 1-2 x 106 cells/mL in PBS or 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge to remove debris. Cell lysates may be assayed undiluted or diluted as necessary in 1X Assay Buffer. • Serum, plasma, saliva, or urine: To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. The supernatant may be assayed directly or diluted as necessary in 1X Assay Buffer. Notes: • All samples should be assayed immediately or stored at -80 °C for up to 1-2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples. • Samples with NADH concentrations above 10 µM and glutathione concentrations above 50 µ M will oxidize the Colorimetric Probe and could result in erroneous readings. To minimize this interference, it is recommended that superoxide dismutase (SOD) be added to the reaction at a final concentration of 40 U/mL (Votyakova and Reynolds, Ref. 2). • Avoid samples containing DTT or β-mercaptoethanol since the Colorimetric Probe is not stable in the presense of thiols (above 10 µM). 4
Assay Procedure:	1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate. 2. Add 50 µL of each lactate standard or unknown sample into wells of a 96-well microtiter plate. 3. Add 50 µL of Reaction Mix to each well. Mix the well contents thoroughly and incubate for 30-45 minutes at 37 °C protected from light. Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics. 4. Read the plate with a spectrophotometric microplate reader in the 540-570 nm range. 5. Calculate the concentration of lactate within samples by comparing the sample OD to the standard curve.
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	RT/-20 °C
Storage Comment:	Upon receipt, store the 10X Assay Buffer at 4°C. Store all remaining components at -20°C. The Colorimetric Probe is light sensitive and must be stored accordingly. Avoid multiple freeze/thaw cycles.

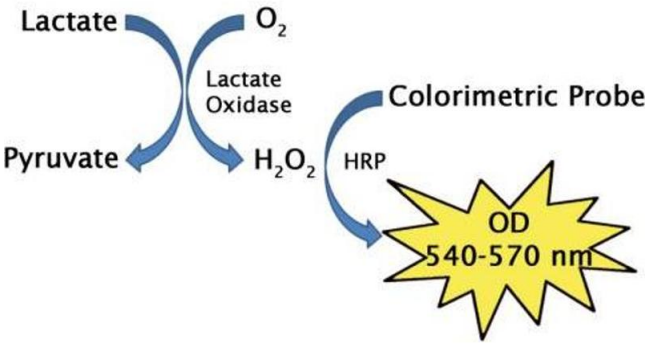
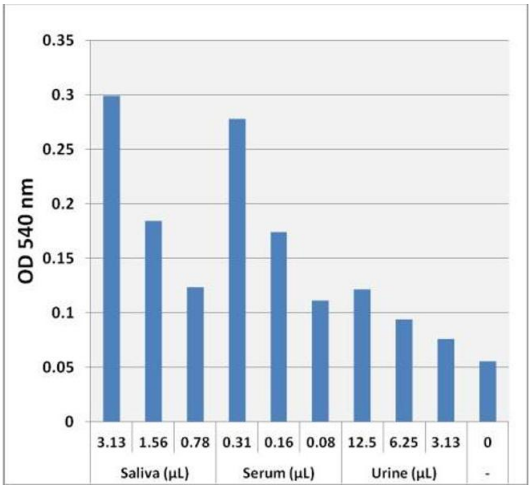
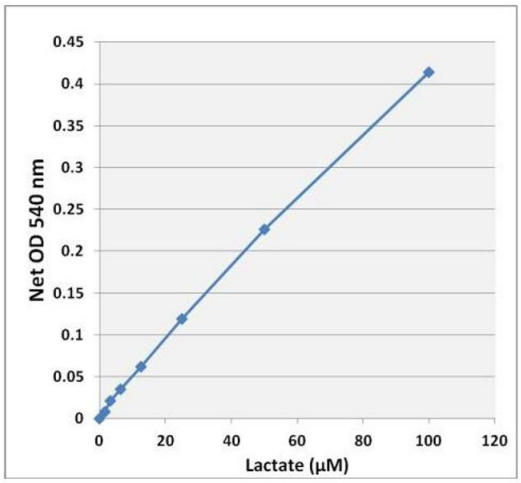


Image 1. Lactate assay principle



Biochemical Assay

Image 2. Lactate detection in human saliva, serum, or urine using the Lactate Assay Kit (Colorimetric).



Biochemical Assay

Image 3. Lactate standard curve