DETERMINATION OF LACTATE CONCENTRATION USING PHOTOPETTE® CELL

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- *Photopette® Cell makes quantification of lactate efficient and easy.*
- *The enzymatic assay can be used for serum, plasma, urine, cell culture/fermentation media or in food & beverages.*

OBJECTIVE

This application note provides an easy and fast enzymatic assay to quantify lactate in biological samples such as serum, plasma, urine, cell culture/fermentation media or in food & beverage products using Photopette®.

INTRODUCTION

During anaerobic glycolysis, energy is produced. In this process, lactate dehydrogenase (LDH) catalyses the oxidation of lactate to pyruvate and an equimolar amount of nicotinamide adenine dinucleotide (NAD^+) is reduced to its reduced form NADH. The amount of NADH produced is directly proportional to the lactate concentration in the sample [1]. The produced NADH is measured by its absorbance at 340 nm.

$$\text{Lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + \text{NADH} + \text{H}^+$$

This enzymatic method is applicable for beverages, food and various biological samples including serum. Photopette® Cell at 340 nm was used to demonstrate the lactate assay for the measurement of unknown lactate concentrations in fermentation media.

MATERIALS AND APPARATUS

Instrument:

- Photopette® Cell with 340 nm wavelength
- Incubator (37 °C)

Reagents:

- L-lactic dehydrogenase (Sigma Aldrich #L3916)
- Glycine buffer (Sigma Aldrich #G5418)
- 10 mg NAD in pre-weighed vials (Sigma Aldrich, #N8285)
- Sodium lactate (Sigma Aldrich, #L7022)

METHOD

EXPERIMENTAL PROCEDURE

Stock solution: A 20 mM lactate stock solution was first prepared by weighting 224 mg dried sodium lactate powder into a 100 mL graduated flask and filling up to the 100 mL mark with Di water. Standard lactate solutions with concentrations between 0.5 mM and 12 mM were prepared by serial dilution of the stock solution in water.

Reaction mixture: Prepare a reaction mixture by pipetting 2 mL of glycine buffer, 4 mL of water and 0.1 mL of L-lactate dehydrogenase into a NAD vial. Mix the vial thoroughly by capping and inverting the vial several times (do not shake).

Protocol: Pipette 1.45 mL of the reaction mixture into 2 mL reaction tubes labelled BLANK, CALIBRATION and SAMPLE. Pipette 50 µL of Di water into the 'BLANK' reaction tube; pipette 50 µL of the standard ethanol solutions into the CALIBRATION reaction tubes and 50 µL of sample into the SAMPLE tubes. Incubate all reaction tubes for 15 minutes at 37 °C.

Turn on the Photopette® Cell device and connect to the Photopette® iOS/Android app. Refer to the Photopette® user manual for operating and safety precautions [2]. Select '340

nm' as the wavelength. Select dataset and set additional settings (if needed) before selecting 'Start Measurement'. Please follow the video tutorials available at www.tipbiosystems.com to get familiar with the measurement process.

Place a CuveTip® firmly onto the Photopette® device probe and insert into the blank sample to perform auto-zero. Ensure that there is no air-bubble trapped in the CuveTip® cavity. Presence of air bubbles may disrupt the optical path and cause errors. A guide to use the CuveTip® correct is available as download [3]. Use the same CuveTip® to measure the standard lactate solutions from low to high lactate concentrations. Ensure that there is no liquid transferred between samples by contacting the CuveTip® with a tissue paper and remove any liquid by capillary action. Repeat the procedure for the samples with unknown lactate concentration.

DETERMINING CONCENTRATION OF AN UNKNOWN SAMPLE

Depending on the estimated unknown lactate concentration of the sample, prepares several dilutions. Serum or plasma samples do not require a dilution. Use the dilutions as duplet or triplet samples as explained above. The lactate concentration is calculated by substituting the absorbance value obtained into the equation of the standard curve and factoring in the dilution factor.

RESULTS AND DISCUSSION

The results of the measurements for the lactate serial dilutions are tabulated in Table 1 below.

Concentration of lactate standard (mM)	Absorbance (AU)	Standard Deviation (SD)
0.0 (blank)	0.000	0.001
0.5	0.098	0.001
2.0	0.367	0.001
4.0	0.594	0.001
8.0	1.052	0.001
12.0	1.536	0.002

Table 1: Absorbance values of lactate standard solutions (average of 5 repeats).

The data was used for the generation of a standard curve, and to determine experimental parameters such as limit of detection and linear range.

STANDARD CURVE

According to the Product Information by Sigma Aldrich for L-Lactic Dehydrogenase from bovine heart (#L3916), the recommended lactate concentration range for the assay is from 0.22 mM to 13.13 mM. A standard curve was plotted in Figure 2 for the data within the range of 0.0 mM to 12 mM lactate. A linear regression was performed on the data using Microsoft Excel® software, and the equation of the standard curve along with its R-squared value was obtained. The slope of the standard curve obtained was 0.125 AU/mM.

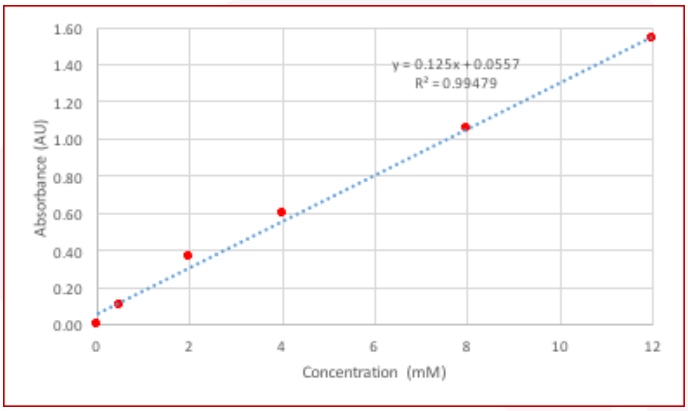

Figure 2: Standard curve for the lactate solutions using Photopette® Cell.

Photopette® users may download a pre-configured worksheet for the lactate analysis from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software and will aid users in generating the standard curve. It is advisable to prepare a complete standard curve with at least 5 data points (lactate concentrations) and a blank for the first measurement to confirm linearity. For follow up measurements, it is advised to run one or two lactate standards together with the unknown samples.

EXPERIMENTAL PAPRAMETERS

Linear range

The calibration curve of Figure 2 shows a linear range to at least 12 mM of lactate.

LIMIT OF DETECTION

The Limit of Detection (LOD) for this measurement using Photopette® is determined by factoring in the standard-deviation for blank measurements as well as experimental data using the equation given below:

$LOD = 3 \times SD_{blank} / Slope_{standard\ curve}$.

Standard Deviation for blank measurements with 50 repeats using same CuveTip® was found to be 0.001 AU. Thus, the limit of detection for the lactate assay with Photopette® using the equation above was 0.024 mM.

LOD = 3 x 0.001 AU / (0.125 AU/mM)

LOD = 0.024 mM

LACTATE CONCENTRATION OF UNKNOWN SAMPLES

An undiluted serum sample was measured and the average of triplets resulted in an absorbance of 0.102. The linear regression of the lactate standards of Figure 2 is:

y = 0.125x + 0.056

Rearrangement to the lactate concentration “x” results in:

x = (y - 0.056) / 0.125

Inserting the absorbance of the unknown sample results in:

x = (0.112 - 0.056) / 0.125

x = 0.448 mM lactate

EXPERIMENTAL VARIATIONS

The same measurement procedure can be used to determine lactate concentrations in other samples such as various beverages, food and biological samples including serum, plasma and fermentation media. For strongly colored samples the absorbance at 340 nm must be taken into account and corrected for. Non-liquid and turbid samples need extraction and/or filtration before the assay can be performed.

LIMITATIONS

The calibration-range was limited by the concentration range recommended by Sigma-Aldrich. Therefore, the range of standard curve is not a limitation of the Photopette® device.

SUMMARY

This application notes guides a user in determining lactate concentrations in various samples by measuring the absorbance at 340 nm. The experiment is easy to conduct in less than 30 min and is low cost. Biological samples such as blood, plasma and cerebrospinal fluid may be used. Certain biological samples must be processed before use in the experiment. The steps for processing the biological samples are highlighted in the Product Information by Sigma Aldrich for L-Lactic Dehydrogenase from bovine heart (#L3916). The method can be easily adapted by breweries, analytical laboratories or for school experiments.

REFERENCES

[1] Sigma Aldrich, "L-Lactic Dehydrogenase from bovine heart #L3916."

[2] Tip Biosystems Pte Ltd, "Photopette® User Manual V1.0.0," *Singapore,* 2017.

[3] Tip Biosystems Pte Ltd, Technical Note “How to use Photopette’s® CuveTip® correctly”