Fluoro Lactate™

L-Lactate Assay Kit

Contact Information

Address	Cell Technology Inc 23575 Cabot Blvd. Suite 203 Hayward, CA 94545 USA
Telephone	650-960-2170
Toll Free	888 7ASSAYS (727-7297)
Fax	650-960-0367
General Information	info@celltechnology.com
Sales	sales@celltechnology.com
Technical Questions	techsupport@celltechnology.com
Website	www.celltechnology.com

Revised protocol 5/06

Notes
Revised protocol 08/12
Update 01/13

Content Table

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

I. Introduction:

Lactate is an intermediate product of carbohydrate metabolism. Of the two forms of Lactate, D- and L-, the L-lactate is the predominant isomer found in biological systems. L-lactate is formed during the anaerobic glycolysis by conversion of pyruvate to L-lactate by lactate dehydrogenase. Lactate level is an indicator for tissue oxygen demand and utilization. Abnormally high lactate levels are associated with diseases such as diabetes and lactate acidosis. Our lactate assay is a lactate oxidase-based method for detecting L-lactate in biological samples such as serum, plasma, blood, urine, and tissue extract.

In the assay, lactate oxidase (LOX) catalyzes the oxidation of L-lactate to pyruvate, along with the concomitant reduction of hydrogen peroxide (H_2O_2). The detection utilizes a non-fluorescent detection reagent, which is oxidized in the presence of horseradish peroxidase (HRP) and LOX to produce its fluorescent analog.

II. Assay Principle:

L-Lactate + O_2 $\xrightarrow{LOX}$ Pyruvate + H_2O_2
H_2O_2 + HRP + Detection Reagent (non-fluorescent) →fluorescent analog ($Ex_{530\text{-}571nm}/Em_{590\text{-}600nm}$)

III. Storage:

1. Short term (several weeks): Kit part# 1 refrigerated at 2-4°C, Kit part# 2 frozen at -20°C and away from light.
2. Long term: see individual components.

IV. Warnings and Precautions:

1. For research use only. Not for use in diagnostic procedures.
2. Practice safe laboratory procedures by wearing protective clothing and eyewear.
4. Glutathione (reduced form GSH) may interfere with the assay.
   See Technical note #3.

V. FLLACT Part # 5062: Kit Contents (for 100 assays):

Kit Part # 1

1. **Part # 7022: Lactate Standard 4mM:** One vial of 100X stock, 500 µl.
2. **Part # 6029: Horseradish Peroxidase:** 18.9 Units of enzyme.
3. **Part # 3011: 5X Reaction Buffer:** 25 ml.

Kit Part # 2

4. **Part # 4026. Detection reagent:** One vial for 100 assays.
5. **Part # 6025. Reaction enzyme mix:** One vial 1.1 ml

Materials required but not supplied:

1. Black 96-well plates
2. Fluorescence plate reader
3. Deionized water

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

VI. Preparation of Reagent Working Solutions:

1. **Prepare 25 ml of 1X Reaction buffer:** 5ml of 5X Reaction buffer, Part # 3011 is added to 20ml of deionized water to make 1X reaction buffer. This should be sufficient for performing 100 assays.

Note: It is important to equilibrate the buffer to room temperature before use as crystals may form on storage. This can be done by warming in a 37ºC water bath or incubator for a few minutes.

2. **Working stock solution of Horseradish Peroxidase Part # 6029:** Quickly spin down the contents of the vial before opening. To the contents of the vial, add 1.89ml of 1X Reaction buffer. This gives a working stock of 10U/ml of HRP.
   Once diluted, the unused HRP should be stored at -20°C as single use aliquots.

3. **Working stock solution of Detection Reagent Part # 4026:** Dissolve the contents of the vial in 110µL of DMSO. Once reconstituted, this should be used promptly and any remaining reagent can be aliquoted and frozen at -20°C. **Avoid repeated freeze thaw cycles.**

4. **Reaction enzyme mix Part # 6025:** It is ready to use. Once it is thawed, it should be aliquoted and frozen at -20°C. Avoid repeated freeze-thaw cycles.

VII. Assay Protocol:

1. To prepare lactate standard curve, a 100X Lactate standard stock at 4mM is supplied with the kit. (Part# **7022)**. Dilute the appropriate amount of 100X lactate stock in 1X Reaction Buffer to make standard curve concentrations ranging from 0 to 40 µM (1X).

   Label suitable tubes 1-8. To tube #1, add 990 µL of 1X Reaction Buffer (Part# **3011**) and 10 µL of the Lactate standard (Part# **7022)**. This will make a 40 µM solution of lactate. Next serially dilute (1:2) the 40 µM solution of Lactate standard in 1X Reaction Buffer (Part# **3011**) to construct a standard curve. This can be accomplished by adding 500 µL of 1X Reaction Buffer (Part# **3011**) into tubes #2-8. From tube #1 remove 500µL of the 40 µM lactate and add it to tube #2 containing 500 µL 1X Reaction Buffer to give 20 µM lactate. Gently vortex tube #2 and pipette out 500 µL from tube #2 and add it to tube#3 containing 1X Reaction Buffer. Continue this process to tube #7. Tube# 8 is the blank control. The final Lactate concentration in the well will be 2.2 times less than in the tube.

Tube #	Lactate concentration in tubes	Final Lactate concentration in wells
1	40 µM	18.18 µM
2	20 µM	9.09 µM
3	10 µM	4.545 µM
4	5 µM	2.27 µM
5	2.5 µM	1.14 µM
6	1.25 µM	0.57 µM
7	0.625 µM	0.28 µM
8	0	0

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

3. Be sure to include a positive and negative control in the assay if a standard curve is not used.

4. Pipette 50µL of the lactate standard dilutions, controls and samples into the wells of a 96-well plate.

   Please see Technical Note# 2.

5. Add 10µL of the reaction enzyme mix to each well to begin the reaction.

6. Incubate for 10 minutes, at RT, away from light.

7. Prepare 5ml **reaction cocktail** (for 100 assays) as follows:
   100µL of working stock solution Detection Reagent
   200µL of working stock solution of HRP
   4.7ml of 1X Reaction buffer

   Add 50µL of the reaction cocktail to each well to begin the reaction.

8. Measure fluorescence at excitation: 530-570nm (570nm is the optimal excitation) and emission 590-600nm in a fluorescent plate reader for 5-20 min.

9. Subtract background fluorescence from each reading.

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

VIII. Data:

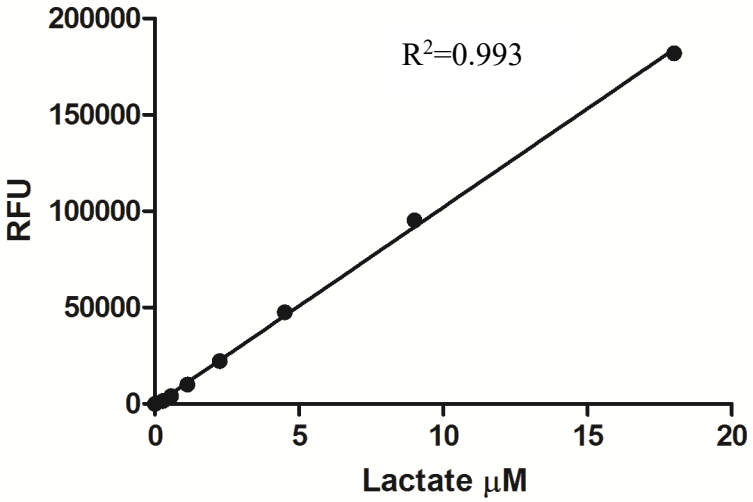


Figure1. Standard curve of Lactate. 50 µl serial dilutions of lactate (Starting dose 40 µM in tubes) were added to the wells of 96-well fluorescent plate. 10 µl reaction enzyme mix was added to each well and incubated plate at 37^0C for 10 min. 50 µl of detection reagent was added and the plate was read at Ex/Em=530/590 nm after incubation for 10 min.

Serum Dilutions	Lactate (µg/ml)
1:10	1.882
1:20	0.920
1:40	0.461
1:80	0.227

Table 1. An example showing serum L-lactate level upon serial dilution. 50µl diluted serum was added to the wells of 96-well fluorescent plate. 10 µl LOX was added to each well and the plate was incubated at 37ºC for 10 min. 50 µl of detection reagent was added and plate was read at Ex/Em=530/590 nm.

Spike and Recovery Results:

Amount Lactate spiked in the serum	% Recovery
40 µM	94%
10 µM	101%

Table 2. Spike and recovery experiments were performed to estimate % recovery of lactate. Serum (1:100) was spiked with lactate with the concentrations mentioned in the table above. The samples were processed as described in the protocol.

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

Amount Lactate spiked in the heat-inactivated serum	% Recovery
40 µM	95%
10 µM	101%

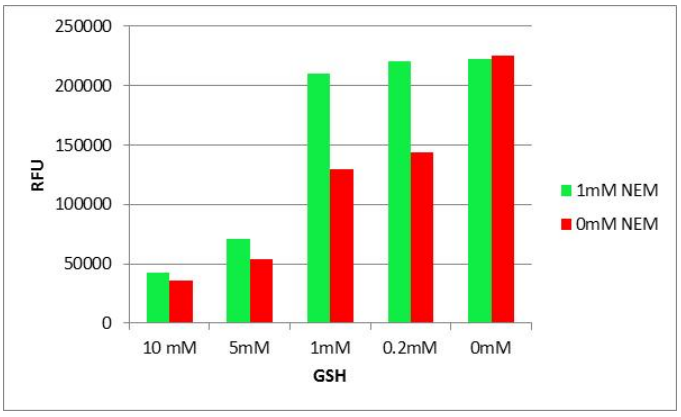
Table 3. Spike and recovery experiments were performed to estimate % recovery of lactate. Serum (1:100, heat-inactivated @ 56ºC, 30 min) was spiked with lactate according to the concentrations mentioned in the table above. The samples were processed as described in the protocol.

IX. Technical Notes:

1. The reaction cocktail once prepared cannot be stored.

2. Preparation of lactate standard curve: It is important to prepare the standard curve in the same matrix or media as your sample. If your samples are in PBS, you can use the supplied 1X Reaction Buffer to construct your standard curve. If your samples are in media, prepare your standard curve in the same media. Serum based media tends to suppress the Fluorescent signal.

3.



As illustrated in above figure, at glutathione (reduced form GSH) concentrations higher than 0.2 mM, detection reagent oxidation results from side chain reaction between GSH and HRP. This could cause aberrant readings. To minimize this interference it is recommended to add N-Ethylmaleimide (NEM) at 40mM to the reaction (4 µM of lactate was used here).

4. The 5X reaction buffer should be equilibrated to room temperature before use as crystals may form upon storage.

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com

References:

1. Hasegawa H., Fukushima T., Lee J., Tsukamoto K., Moriya K., Ono Y. and Imai K. (2003) Determination of serum D -lactic and L -lactic acids in normal subjects and diabetic patients by column-switching HPLC with pre-column fluorescence derivatization. *Anal Bioanal Chem* 377:886-891.
2. Kondoh Y., Kawase, M. and Ohmori S. (1992) Concentration of D-Lactate and its metabolic intermediates in liver, blood, and muscle of diabetic and starved rats. *Res Exp Med* 192: 407-414.
3. Lin, C. Y., Chen S. H., Kou G. H., Kuo C. M. (1999) An Enzymatic Microassay for Lactate. Concentration in Blood and Hemolymph. *Acta Zoologica Taiwanica* 10: 91-101.
4. McLellan, A. C., Phillips, S. A., and Thornally, P. J. (1992) Flourimetric assay of D-lactate. *Anal Biochem* 206: 12-16.
5. Scheijen J.L., Hanssen N. M., van de Waarenburg M. P., Jonkers D. M., Stehouwer C. D., Schalkwijk C. G. (2012) L(+) and D(-) lactate are increased in plasma and urine samples of type 2 diabetes as measured by a simultaneous quantification of L(+) and D(-) lactate by reversed-phase liquid chromatography tandem mass spectrometry. *Exp Diabetes Res*. 2012(doi:10.1155/2012/234812).
6. White R., Yaeger D., and Stavrianeas S. Determination of Blood Lactate Concentration: Reliability and. Validity of a Lactate Oxidase-Based Method (2009) *Int. J. Exerc Sci* 2: 83-93.

Cell Technology, Inc. · 23575 Cabot Blvd., Suite 203 · Hayward, CA 94545 USA

Toll Free in USA 877-727-7297 · Phone 650-960-2170 · Fax 650-960-036www.celltechnology.com