ab174096 – D-Lactate Activity Assay Kit (Fluorometric)

For the measurement of D-Lactate enzymatic activity in various tissues, cells, and biologic fluids.

For overview, typical data and additional information please visit: www.abcam.com/ab174096
(use www.abcam.cn/ab174096 for China, or www.abcam.co.jp/ab174096 for Japan)

Storage and Stability: Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt if components have not been reconstituted.
Reconstituted components are stable for 2 months.
Aliquot components in working volumes before storing at the recommended temperature.
Avoid repeated freeze-thaws of reagents.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer XII	25 mL	-20 °C	4 °C
PicoProbe I	400 µL	-20 °C	-20 °C
Enzyme Mix XIII	1 vial	-20 °C	-20 °C
Developer Solution X	1 vial	-20 °C	-20 °C
D-Lactate Standard (100 mM)	100 µL	-20 °C	-20 °C

Materials Required, Not Supplied

Microplate reader capable of measuring fluorescence in kinetic mode
White 96 well plate with flat bottom

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

1. **PicoProbe I:** Ready to use as supplied. Warm to temperature before use. store at -20°C.
2. **Enzyme Mix XIII:** Reconstitute with 220 µL of Assay Buffer XII. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw and keep on ice while in use. Stable for 2 months at -20°C.
3. **Developer Solution X:** Reconstitute with 220 µL of Assay Buffer XII. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw and keep on ice while in use. Stable for 2 months at -20°C.
4. **D-Lactate Standard:** Provided as 100 mM stock solution. Store at -20°C, stable for at least 3 freeze/thaw cycles.

Sample Preparation

1. Prepare 20-50 µL test samples in a 96-well plate (for high target concentrations, dilute the samples). Bring the volume to 50 µL/well with Assay Buffer XII. We suggest using different volumes of your sample to ensure the readings are within the standard curve range.

ΔNOTES:

- **(a)** Tissue (20 mg) or cells (2 x 10^6) can be homogenized in 100 µL of Assay Buffer XII. Centrifuge at 10,000x g for 10 minutes to remove insoluble material. Soluble fractions may be assayed directly.
- **(b)** Body fluids, such as urine, should be filtered using a 10 kDa MW spin filter. Dilute samples with pure water. An appropriate dilution factor is usually between 1:10 and 1:100. Diluted samples can be assayed directly.
- **(c)** Food samples. A) Beer: Remove CO_2 by vacuum-filtering samples. Dilute samples with pure water (appropriate dilution factor is 1:10). Diluted samples can be assayed directly. B) Yogurt: Vortex 1 gram of yogurt in 10 mL of water until homogeneous. Dilute with pure water (dilution factor of ~ 1:100 to 1:500). Diluted samples can be assayed directly.
- **(d)** Endogenous enzyme activity may cause loss of D-Lactate. Samples containing enzyme activity (such as culture medium or tissue lysate) should be kept at -80°C or filtered through a 10 kDa MW spin filter.

D-Lactate Standard Preparation

Always prepare a fresh set of standards for every use & discard working standard dilutions after use as they do not store well.

1. Dilute D-Lactate Standard to 1 mM (1000 pmol/ µL) by adding 10 µL of 100 mM D-Lactate Standard to 990 µL of Assay Buffer XII and mix well.
2. Dilute standard further to 0.02 mM (20 pmol/ µL) by adding 20 µL of the 1 mM D-Lactate Standard stock to 980 µL of Assay Buffer XII and mix well. Use this 0.02 mM stock for the assay.
3. Add 0, 2, 4, 6, 8, and 10 µL of the 0.02 mM D-Lactate Standard into a series of wells in a 96 well white plate.
4. Adjust the volume to 50 µL/well with Assay Buffer XII to generate 0, 40, 80, 120, 160, and 200 pmol/well of D-Lactate Standard.

ΔNOTE: To improve accuracy and reduce CVs, the standard curve may be prepared in duplicate, in a microplate or microcentrifuge tubes using the table below. Each standard mix has enough volume to set up duplicate readings (2 x 50 µL).

Standard #	0.02 mM D-Lactate Standard (µL)	Assay Buffer XII (µL)	Final volume standard in well (µL)	D-Lactate in well (pmol)
1	0	125	50	0
2	5	120	50	40
3	10	115	50	80
4	15	110	50	120
5	20	105	50	160
6	25	100	50	200

Assay Procedure

1. Make enough Reaction Mix and Background Control Mix for the number of assays to be performed, 50 µl per reaction as shown below. Remember to account for the Standard Curve wells when calculating the amount of Reaction Mix needed.

ΔNOTE: For samples having high NADH levels, add 50 µL of Background Control Mix to sample background control well(s) and mix well.

Component	Reaction Mix (µL)	Background Control Mix (µL)
Assay Buffer XII	45	47
PicoProbe I	1	1
Enzyme Mix XII	2	-
Developer Solution X	2	2

2. Add 50 µL of the Reaction Mix to Each well containing the D-Lactate Standard and Test Sample(s). Gently mix by pipetting up and down
3. Add 50 µL of the Background Control Mix to each Sample Background Control well. Gently mix by pipetting up and down.
4. Immediately measure fluorescence (Ex/Em = 538/587 nm) for 60 minutes at 37°C in a microplate reader with kinetics mode.

Data analysis

1. For the D-Lactate Standard Curve, subtract the 0 pmol/well Standard fluorescence reading from all the Standard readings ($\Delta RFU = RFU_{standard} - RFU_{blank}$). Plot the background-corrected Standard values and calculate the slope of the D-Lactate Standard Curve.
2. For Sample Reaction Wells (including paired Sample Background Control wells), choose any two time points (T_1 and T_2) in the linear phase of the reaction progress curves. Obtain the corresponding fluorescence values at those points (A_1 and A_2) and determine the change in fluorescence over the time interval: $\Delta A = A_2 - A_1$.
3. Subtract the Sample Background Control (ΔA_{BC}) from the corresponding Sample (ΔA_S) to obtain the net change in fluorescence: $\Delta A_{NET} = \Delta A_S - \Delta A_{BC}$.
4. The net values (ΔA_{NET}) are applied to the Standard Curve to get *B* pmol of substrate metabolized during the reaction time.
5. To determine the Sample D-Lactate Activity, use the following equation:

$$\textbf{Sample D-Lactate Activity} = \frac{\boldsymbol{B}}{\boldsymbol{\Delta T \times V}} \times \boldsymbol{D} = pmol/\mu L = nmol/mL$$

Where:
$\boldsymbol{B}$ is the D-Lactate amount (pmol) from Standard Curve
$\boldsymbol{V}$ is the sample volume (µL) added to the well
$\boldsymbol{D}$ is the sample dilution factor (if applicable, *D* = 1 for undiluted samples)

ΔNOTE: Remember to account for any dilution of the sample made during sample preparation, before adding sample to the sample well, when determining *D*.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)