

**Technical Manual**

**FluoroDazzle D-Lactate Assay Kit**

**Catalogue Code: BA0100**

**Pack Size: 100 assays**

**Research Use Only**

## DESCRIPTION

Lactate is generated by lactate dehydrogenase (LDH) under hypoxic or anaerobic conditions. Monitoring lactate levels is, therefore, a good indicator of the balance between tissue oxygen demand and utilization and is useful when studying cellular and animal physiology. D-Lactate is produced in only minor quantities in animals and measuring for D-lactate in animal samples is a means to determine the presence of bacterial infection.

Simple, direct and automation-ready procedures for measuring lactate concentration are very desirable. The Assay Genie FluoroDazzle D-Lactate Assay Kit is based on lactate dehydrogenase catalyzed oxidation of lactate, in which the formed NADH reduces a probe into a highly fluorescent product. The fluorescence intensity of this product, measured at  $\lambda_{ex/em} = 530/585$  nm, is proportional to the lactate concentration in the sample.

## APPLICATIONS

**Direct Assays:** D-lactate in serum, plasma, urine, cell media samples and other biological samples.

## KEY FEATURES

**Sensitive and accurate.** Detection limit of 1 mM and linearity up to 50 mM D-lactate in 96-well plate assay.

**Convenient.** The procedure involves adding a single working reagent, and reading the fluorescence after 60 min. Room temperature assay.

**High-throughput.** Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

## KIT CONTENTS

<b>Assay Buffer:</b>	10 mL	<b>Enzyme A:</b>	120 $\mu$ L
<b>NAD Solution:</b>	1 mL	<b>Enzyme B:</b>	120 $\mu$ L
<b>Probe:</b>	750 $\mu$ L	<b>Standard:</b>	1 mL

**Storage conditions.** The kit is shipped on ice. Store all reagents at  $-20^{\circ}\text{C}$ . Shelf life: 6 months after receipt.

**Precautions:** reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## SAMPLE PREPARATION AND CONSIDERATIONS

The following substances interfere and should be avoided in sample preparation: EDTA ( $>0.5$  mM), ascorbic acid, SDS ( $>0.2\%$ ), sodium azide, NP-40 ( $>1\%$ ) and Tween-20 ( $>1\%$ ). Samples suspected of having endogenous L-LDH enzyme activity (e.g. serum, plasma, culture medium with FBS, etc.) should be deproteinated using a 10 kDa spin filter (e.g. Microcon YM-10). Deproteinated serum should be diluted 3  $\times$  with  $\text{dH}_2\text{O}$ . Samples containing higher than 50  $\mu$ M pyruvate require an internal standard.

## PROCEDURES

- Standard Curve.** Prepare 1000  $\mu$ L 40 mM D-lactate Premix by mixing 2  $\mu$ L 20 mM Standard and 998  $\mu$ L distilled water. For cell culture samples, prepare 1000  $\mu$ L 40 mM D-lactate Premix by mixing 2  $\mu$ L 20 mM Standard and 998  $\mu$ L culture medium without serum. Dilute standard as follows.

No	Premix + $\text{H}_2\text{O}$ or Medium	D-Lactate (mM)
1	100 $\mu$ L + 0 $\mu$ L	40
2	60 $\mu$ L + 40 $\mu$ L	24
3	30 $\mu$ L + 70 $\mu$ L	12
4	0 $\mu$ L + 100 $\mu$ L	0

Transfer 50  $\mu$ L standards into wells of a black, flat bottom 96-well plate.

**Samples.** Add 50  $\mu\text{L}$  sample to two separate wells. Set up two reactions for each sample: one with added Enzyme A (Sample) and a No Enzyme A control (Sample Blank).

Samples requiring an internal standard, will need three separate reactions: 1) Sample plus Standard, 2) Sample alone and 3) Sample Blank. For the internal standard first prepare 400  $\mu\text{L}$  250  $\mu\text{M}$  D-lactate standard by mixing 5  $\mu\text{L}$  20 mM Standard and 395  $\mu\text{L}$  dH<sub>2</sub>O. For the Sample plus Standard well, add 5  $\mu\text{L}$  250  $\mu\text{M}$  D-lactate and 45  $\mu\text{L}$  sample. For the Sample and Sample Blank wells, add 5  $\mu\text{L}$  dH<sub>2</sub>O and 45  $\mu\text{L}$  sample.

2. Reagent Preparation. Spin the Enzyme tubes briefly before pipetting. For each Sample and Standard well, prepare Working Reagent by mixing 40  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme A, 1  $\mu\text{L}$  Enzyme B, 10  $\mu\text{L}$  NAD and 5  $\mu\text{L}$  Probe. Fresh reconstitution is recommended. For the Sample Blanks, the Working Reagent includes 40  $\mu\text{L}$  Assay Buffer, 1  $\mu\text{L}$  Enzyme B, 10  $\mu\text{L}$  NAD and 5  $\mu\text{L}$  Probe (**NO Enzyme A**).
3. Reaction. Add 50  $\mu\text{L}$  Working Reagent per reaction well quickly. Tap plate to mix briefly and thoroughly. Incubate for 60 min at RT protected from light.
4. Read fluorescence  $I_{\text{ex/em}} = 530/585 \text{ nm}$ .

### CALCULATION

Plot the D-lactate Standard Curve and determine its slope. The D-lactate concentration of the sample is computed as follows:

$$[\text{D-Lactate}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{\text{Slope } (\mu\text{M}^{-1})} \times n \quad (\mu\text{M})$$

where  $F_{\text{SAMPLE}}$  and  $F_{\text{BLANK}}$  are the fluorescence intensity values of the Sample and Sample Blank respectively. Slope is the slope of the standard curve and  $n$  is the dilution factor (e.g.  $n = 3$  for serum samples).

$$[\text{D-Lactate}] = \frac{F_{\text{SAMPLE}} - F_{\text{BLANK}}}{F_{\text{STANDARD}} - F_{\text{SAMPLE}}} \times 27.8 \quad (\mu\text{M})$$

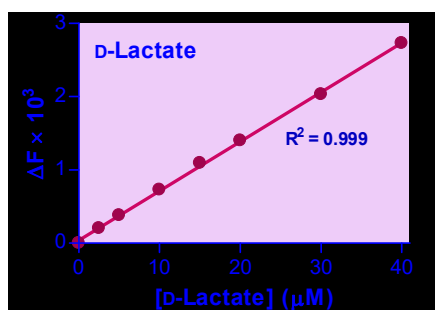
If an internal standard was needed, the sample D-lactate concentration is computed as follows:

where  $F_{\text{SAMPLE}}$  and  $F_{\text{BLANK}}$  are the fluorescence intensity values of the Sample and Sample Blank respectively and  $F_{\text{STANDARD}}$  is the fluorescence intensity value of the Sample plus Standard.

Note: if the sample DF value is higher than the DF for 40  $\mu\text{M}$  D-lactate standard or greater than the DF for the internal standard, dilute the sample in water and repeat the assay. Multiply the results by the dilution factor.

### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting (multi-channel) devices. Black, flat bottom 96-well plates and fluorescent plate reader capable of reading at  $I_{\text{ex/em}} = 530/585 \text{ nm}$ .



Standard Curve in 96-well plate assay in water.

**LITERATURE**

1. Ewaschuk JB et al (2005). D-lactate in human and ruminant metabolism. J Nutr. 135(7):1619-25.
2. Mack DR (2004). D(-)-lactic acid-producing probiotics, D(-)-lactic acidosis and infants. Can J Gastroenterol. 18(11): 671-5.
3. Uribarri J et al (1998). D-lactic acidosis. A review of clinical presentation, biochemical features, and pathophysiologic mechanisms. Medicine (Baltimore) 77(2):73-82.

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