Potential biomarkers in the urine of myocardial infarction rats: a metabolomic method and its application

Peng Jiang[1], Weixing Dai[1], Shikai Yan[2], Zhongliang Chen[3], Ruilin Xu[3], Jianmi Ding[3], Li Xiang[1], Shuping Wang[1], Runhui Liu[1,*], Weidong Zhang[1, 2,**]

1. The process of detecting LDH level in rat serum

1.1 Adding the following reagents into detector tube one by one according to instruction manual:

	Blank (ml)	Standard (ml)	Test (ml)	Control (ml)
Distilled water	0.07	0.05		0.05
2 mmol/L Pyruvate standard solution		0.02		
Serum sample			0.02	0.02
Base buffer solution	0.25	0.25	0.25	0.25
Coenzyme□			0.05	
Vortex-mixing and then placing into water bath at 37 °C for 15 min				
2,4-Dinitrobenzonitrile	0.25	0.25	0.25	0.25
Vortex-mixing and then placing into water bath at 37 °C for 15 min				
0.4 mol/L NaOH solution	2.5	2.5	2.5	2.5

Vortex-mixing and waiting for 3 min at room temperature, then setting the detector to zero at 440 nm using distilled water and then detecting the samples' optical density (OD)

1.2 Calculating formula

The activity of LDH in serum (U/L) = $[(OD_T-OD_C)/(OD_S-OD_B)]\times C_S\times 1000$

OD_T—the OD value of serum sample

OD_B—the OD value of blank sample

OD_S—the OD value of standard sample

OD_C—the OD value of control sample

C_S—the concentration of pyruvate standard solution (2mmol/L)；

2. The process of detecting CK level in rat serum

2.1 Adding the following reagents into detector tube one by one according to

instruction manual: (All the reagents and samples should be pre-heated in a water bath at 37 °C for 15 min.)

	Test (μl)	Control (μl)
Serum	20	
Reagent 1	80	80
Reagent 2	20	20
Reagent 3	50	50
Reagent 4	100	100
Reagent 5	50	50
Vortex-mixing for 30s and then placing into water bath at 37 °C for 20 min		
Reagent 6	100	100
Serum		20
Vortex-mixing for 30s and then centrifuged at 3500 rpm for 10 min		
Supernatant	300	300
Phosphate determination reagent	2000	2000

Vortex-mixing and placing into water bath at 45 °C for 15 min, then setting the detector to zero at 660 nm using distilled water and detecting the samples' optical density (OD).

2.2 Calculating formula

The standard curve of the activity of CK (Y=7.4491X-0.0716) has been given in the instruction manual.

X—the OD value of serum sample with background subtraction

Y—the activity of CK (U/ml)

The activity of CK in serum (U/L) = $[7.4491\times (OD_T-OD_C)-0.0716] \times 1000$

OD_T—the OD value of serum sample

OD_C—the OD value of control sample