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Malate dehydrogenase (MDH, EC 1.1.1.37) is an enzyme which is widely present in animal, plant and microbial sources and is conventionally used in clinical tests. MDH catalyzes the reversible reduction of oxaloacetate to malate in the presence of NADH. Malate dehydrogenase is also involved in gluconeogenesis, the synthesis of glucose from smaller molecules. Its existence as separate isoenzymes in the cytosol and in the mitochondria is associated with the function of a shuttle system (the malate aspartate shuttle) for the transport of reducing equivalents from the cytosol into the mitochondria.

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-Malate} + NAD^+$$

The disappearance of NADH is measured at 340nm by spectrophotometry.

Malate dehydrogenase (MDH)

A new generation of malate dehydrogenase was recently produced by CUSAg. The enzyme can be used for aspartate aminotransferase assay, which is a simple, rapid, sensitive and homogeneous assay that can be performed using automated systems for high-throughput assays.

Properties	Specification
Appearance	White amorphous powder, lyophilized
Activity	≥300 U/mg -solid or more
Genus	Microorganism
Purification method, Purity	Chromatography, etc, >90% (SDS-PAGE)
Stability	Store at -20°C
Optimum pH	7.5-8.0
Optimum temperature	60°C
pH Stability	pH 3.0-9.0 (37°C, 30 min)
Thermal stability	≤65°C (pH 7.5, 30 min)
Inhibitors	EDTA-2K, Tween-20, NaN_3, etc (activator)
Catalog Number	CSB-DE010

MDH

1 Thermal stability

◎ **Thermal stability of MDH-solid:** Three batches of MDH-solid were stored at -20°C and 37°C for 14 days, respectively. During this period, enzyme activity of MDH was determined on our biochemistry platform.Table.1 shows that there is no significant difference between MDH residual activities of each batch, representing that our CUSAg enzyme was stable from -20 °C to 37°C.

Table.1 Thermal stability of MDH-solid

Batch	Specific activity (U/mg, 14 days)		Relative bias (%)
	-20℃	37℃	
1	1039.9	991.1	-4.7
2	1021.7	1057.3	+3.5
3	999.5	982.7	-1.7

◎ **Thermal stability of aspartate aminotransferase reagent:** CUSAg in-house aspartate aminotransferase reagents prepared with our malate dehydrogenase were respectively stored at 4°C and 37°C for 14 days. During the incubation time, the control (42 U/L) was tested. Table.2 shows our reagent was stable no matter stored at 4°C or 37°C.

Table.2 Thermal stability of aspartate aminotransferase reagent

Batch	△ABS (14 days)		CV(%)
	4℃	37℃	
1	-76.5	-71.5	-6.5
2	-75.5	-76.5	+1.3
3	-74.0	-77.0	+4.1

2 Clinical comparison

Our CUSAg aspartate aminotransferase reagent was also evaluated in medium-scale clinical trials with random blood samples from donations (n=80). Fig.1 shows that the correlation coefficient (r) is as high as 0.99 between in-house biochemistry assay and commercial assay. These results show good agreement between the two systems.

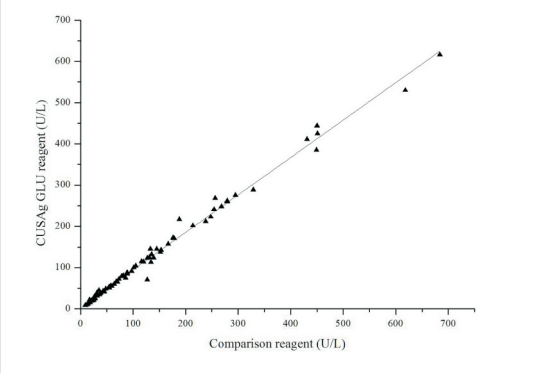

Fig.1 Clinical comparison of in-house aspartate aminotransferase reagent and commercial kit

3 Accuracy

Two levels of aspartate aminotransferase control were analyzed in replicates of three using our two lots of reagents on the CUSAg biochemistry platform. Data from this study are summarized in the below table.3, the relative bias was <4%.

Table.3 Accuracy of aspartate aminotransferase reagent

Control	Control Target value (U/L)	Determined Con. (U/L)			Mean Con. (U/L)	Bias (%)
1	38.0	39.0	39.0	40.0	39.3	3.5
2	149.0	149.0	150.0	150.0	149.7	0.4

4 Precision

Two members of serum samples based panel were assayed, using a single lot of reagents, in replicates of ten on the CUSAg biochemistry platform (from 8 to 40 U/L as normal). As shown in table.4, the system shows excellent precision with CV≤6%.

Table.4 Precision profile of aspartate aminotransferase reagent

Panel Member	n	Mean Con.(U/L)	SD	%CV
1	10	13.5	0.7	5.2
2	10	544.6	1.8	0.3

5 Enzymatic properties

◎ **Optimum pH:** Enzyme activity of MDH was determined at 37°C in different kinds of buffer (100 mM buffer: pH 5.5-8.0 K-phosphate; pH 7.5-8.5 Tris-HCl; pH 8.0-9.0 Borate). As shown in Fig.2, the optimum pH of MDH is from 7.5 to 8.0.

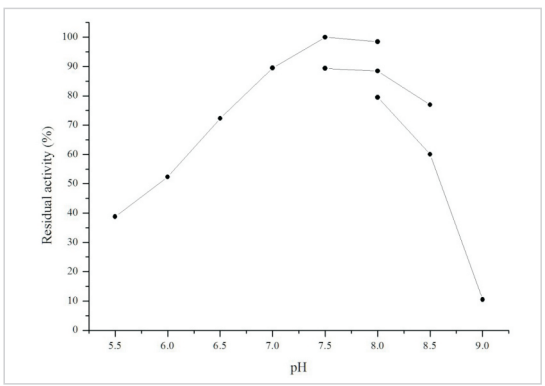

Fig.2 Optimum pH

(37°C in the 100mM buffer solution: pH 5.5-8.0 K-phosphate; pH 7.5-8.5 Tris-HCl; pH 8.0-9.0 Borate)

MDH

◎**Optimum temperature:** The enzyme activity of CUSAg MDH was determined at different temperatures in 100 mM K-phosphate pH 7.5. Fig.3 shows that the optimum temperature of MDH is 60°C.

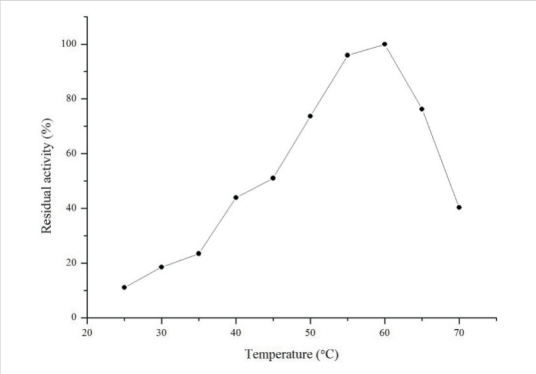

Fig.3 Optimum temperature

(100 mM K-phosphate, pH 7.5)

◎ **pH Stability:** 15 U/mL of CUSAg MDH was incubated at 37°C for 30 minutes in different buffer (100 mM buffer: pH 2.0-3.5 Glycine-HCl; pH 4.0-5.0 Na-phosphate-citrate; pH 5.5-8.0 K-phosphate; pH 8.0-9.0 Tris-HCl; pH 9.5-10.5 CAPS-NaOH; pH 11.0-12.0 Na-phosphate-NaOH), and the residual activities were determined with biochemistry assay. As shown in Fig.4, MDH is stable from pH 3.0 to 9.0.

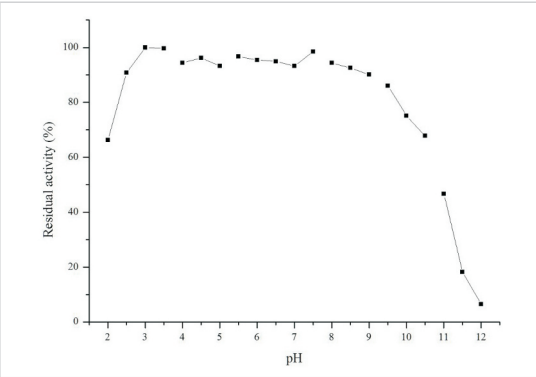

Fig.4 pH stability

(37°C,30 min-treatment in the 100m M buffer solution:
pH 2.0-3.5 Glycine-HCl; pH 4.0-5.0 Na-phosphate-citrate;
pH 5.5-8.0 K-phosphate; pH 8.0-9.0 Tris-HCl;
pH 9.5-10.5 CAPS-NaOH; pH 11.0-12.0 Na-phosphate-NaOH;
enzyme concentration: 15 U/mL)

◎ **Thermal stability:** Our CUSAg MDH was incubated from 25°C to 70°C for 30 minutes in 100 mM K-phosphate buffer (pH 7.5), containing 20 U/mL of MDH, and the residual activities were determined with colorimetric assay on bio-chemical analyzer. As shown in Fig.5, MDH is stable below 65°C.

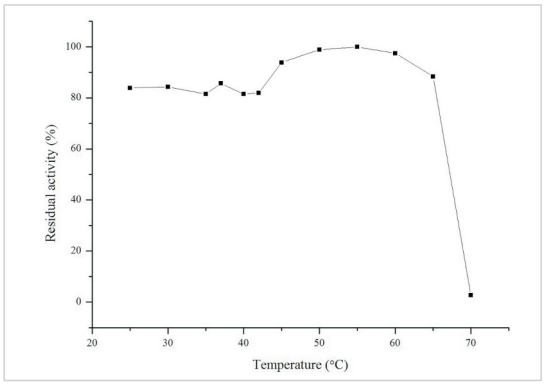

Fig.5 Thermal stability

(30 min-treatment with 100mM K-phosphate buffer,
pH 7.5, enzyme concentration: 20 U/mL)

◎**Effect of Various Chemicals:** CUSAg MDH was dissolved at concentration of 20 U/mL in 100 mM, pH 7.5, Tris-HCl buffer, which was incubated with each chemical at 37°C for 15 min. The activities were determined with biochemistry assay. As shown in table.5, MDH can be activated by EDTA-2K, Tween-20 and NaN_3.

Table.5 Effect of Various Chemicals on MDH

Chemical	Concn.(mM)	Residual activity (%)
None	—	100
$CuSO_4$	5.0	91
$CaCl_2$	5.0	96
$MgSO_4$	5.0	97
$MnCl_2$	5.0	98
NaCl	5.0	94
$NiCl_2$	5.0	96
KCl	5.0	100
$FeCl_3$	5.0	95
NaN_3	5.0	131
Triton X-100	5.0	95
Tween-20	5.0	130
EDTA-2K	5.0	134

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