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Aspartate Aminotransferase/Glutamic Oxaloacetic Transaminase（AST/GOT）Assay Kit Instruction

CAT/NO.: BC006 (Microplate method)

1. Principle:

AST/GOT can make α-oxoglutarate and aspartic acid exchange their amino-group and ketone group, produce glutamic acid and oxaloacetic acid. Oxaloacetic acid can decarboxylate automatically to pyruvic acid. Pyruvic acid reacts with 2,4-dinitro-phenylhydrazine, produces 2,4-dinitrophenylhydrazone. 2,4-dinitrophenylhydrazone appears red brown in alkaline solution. Measure OD values and check standard curve, then it is able to calculate enzyme's activity units.

2. Reagents' composition and preparation(96T):

(1) AST matrix solution: 5ml×1 bottle, can be stocked at 4°C for 6 months;

(2) 2,4-dinitro-phenylhydrazine solution: 5ml×1 bottle, can be stocked at 4°C for 6 months;

(3) 4mol/L NaOH solution: 5ml×1 bottle, can be stocked hermetically at room temperature for 6 months; dilute this solution with distilled water at ratio of 1:9 to make 0.4mol/L NaOH solution before use, how much you need , how much you make, it can be stocked hermetically at room temperature;

(4) 2μmol/ml sodium pyruvate standard solution: 1 tube, can be be stocked at 4°C for 6 months.

(5) 0.1mol/L phosphate buffer: 1 tube, can be can be be stocked at 4°C for 6 months.

3. Operation table:

	Assay well	Contrast well
Matrix solution（μl）already pre -warmed at 37°C	20	20
Sample to assay（μl）	5	

When add sample to each assay well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 30 minutes.

2,4-dinitro-phenylhydrazine solution（μl）	20	20

Sample to assay（μl）		5

When add sample to each contrast well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 20 minutes.

0.4mol/L NaOH solution（μl）	200	200

Shake 96-well plate softly and horizontally to mix well, place at room temperature for 15 minutes, use ELIASA to measure OD values of wells at 510 nm, according to $OD_{Absolute} = OD_{Assay} – OD_{Contrast}$, check standard curve to acquire corresponding AST/GOT activity units.

4. Announcements：

1. Generally, the amount of endogenous ketonic acid in serum sample is very low, so serum contrast tube's absorbance is similar to reagent blank tube's absorbance (Use distilled water to instead of blood serum in reagent blank tube, other operations are same). Therefore, when you assay a batch of samples, it needn't to make serum contrast tube for every sample, you can use reagent blank tube instead, but it needs to make contrast tube for every sample of heavy lipidemia, jaundice and haemolysis.
2. If enzyme activity is higher than 150 units, then dilute sample with physiological saline and assay again.
3. After add 2,4- dinitro-phenylhydrazine solution, please mix well to make reaction complete, methods to add NaOH solution should be same, diifferent methods can cause ddeviations of OD values.
4. In colorimetry, there are commonly used Reitman-Frankel's method and King's method. Unit values decided by standard curve of Reitman-Frankel's method are acquired by contrasting assay between experimental method and Carmen's spectrophotometry (velocity method). It is relatively accurate to report results by Carmen's unit. Definition of Carmen's unit: take 1ml blood serum, reaction solution's volume is 3ml, measure absorbance in cuvette of 1cm light path at 340nm, pyruvic acid produced in 1 mintute at 25°C oxidates NADH to NAD+, absorbance decreasing caused by this oxadation per 0.001 is considered as 1 unit (1 Carmen's unit = 0.482 IU/L, 25°C).

5. AST in blood serum can be stocked at room temperature (25°C) for 2 days, at 0～4°C for 1 week, at 25°C for 1 month.

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Appendix Ⅰ： AST Standard Curve

Standard cure preparation：

	0	1	2	3	4
0.1mol/L phosphate buffer（μl）	5	5	5	5	5
2μmol/mlsodium pyruvate standard solutio（μl）	0	2	4	6	8
Matrix buffer（μl）	20	18	16	14	12
2,4-dinitro-phenylhydrazine solution（μl）	20	20	20	20	20

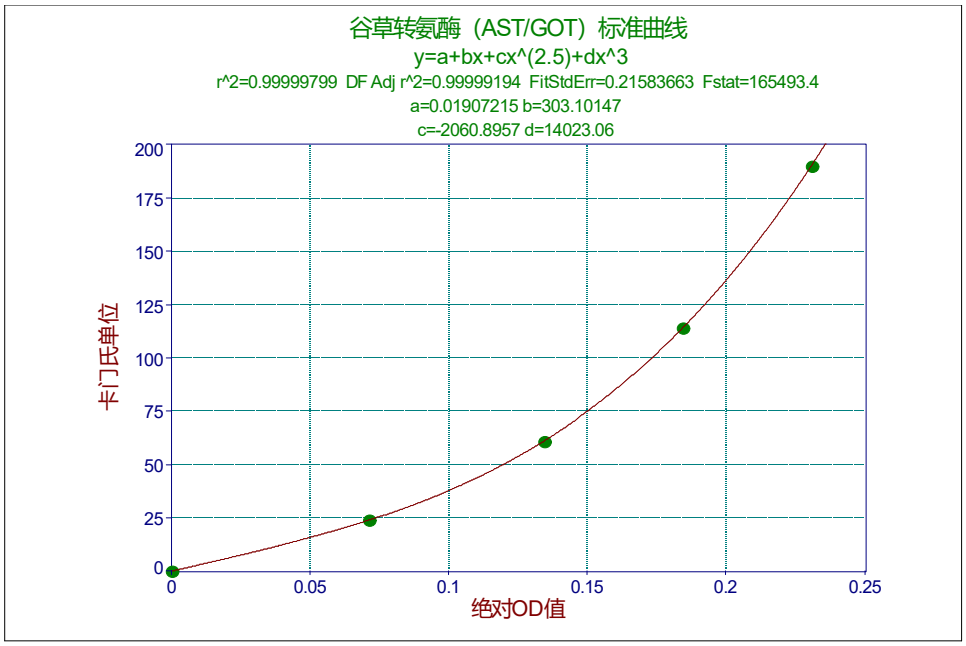
When add standard to each well, please insert tip to liquid at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 20 minutes.

0.4mol/L NaOH solution（μl）	200	200	200	200	200

Shake 96-well plate softly and horizontally to mix well, place at room temperature for 15 minutes, use ELIASA to measure OD values of wells at 510 nm. Use $OD_{Absolute}$ values as abscissaes ($OD_{Absolute} = OD_{Assay} – OD_{Contrast}$,), use corresponding Carmen's units as ordinates, draw coordinate graph fitting to formula, calculate AST activitis in samples by formula in Excel.

Appendix: Referenced standard curve:

OD values in Institute experiments	0.2272	0.2985	0.3616	0.4117	0.4582
$OD_{Absolute}$ values Institute experiments	0	0.0713	0.1344	0.1845	0.2310
Corresponding enzyme activities in Carmen's unit	**0**	**24**	**61**	**114**	**190**



Appendix II： Tissue AST Assay

1. Sample pretreatments:

Weigh tissue accurately, add 9 times (according to mass/weight ratio) physiological saline to make 10% homogenate, centrifuge at 3500rpm for 10 minutes, take supernatant to assay **(AST content in liver tissue is relatively high, so generally it need to dilute with physiological saline to 1% homogenate to assay).**

2. Operation table:

	Assay well	Contrast well
Matrix solution（μl）already pre-warmed at 37°C	20	20
Sample to assay（μl）	5	

When add sample to each assay well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 30 minutes.

2,4-dinitro-phenylhydrazine solution（μl）	20	20
Sample to assay（μl）		5

When add sample to each contrast well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 20 minutes.

0.4mol/L NaOH solution（μl）	250	250

Shake 96-well plate softly and horizontally to mix well, place at room temperature for 15 minutes, use ELIASA to measure OD values of wells at 510 nm, according to $OD_{Absolute} = OD_{Assay} – OD_{Contrast}$, check standard curve to acquire corresponding AST/GOT activity units.

2. Calculating formula and example

(1) Formula:

$$\text{Tissue AST activity (U / gprot)} = \text{AST activity acquired by standard curve} \div \text{Protein concentration in homogenate to assay (gprot / L)}^*$$

Note: gprot / L = gram protein per liter

(2) Example:

Take a piece of mouse liver tissue, make 10% homogenate at mass-weight ratio of 1:9, dilute homogenate with physiological saline to 1%, take 5μl sample and start operations on 96-well plate according to table above, use ELIASA to measure absorbances at 510nm.In results, OD_{Assay} is 0.3594, $OD_{Contrast}$ is 0.2175, protein concentration in 1% mouse liver homogenate is 1.028gprot/L, $OD_{Absolute} = OD_{Assay} – OD_{Contrast}$ = 0.1419, substitute in fitting formula acquired by standard curve, calculate as follows:

AST activity in mouse liver tissue（U/gprot）=67.4646 Carmen's unit ×0.482÷1.028（gprot/L）

=31.6322（U/gprot）

Appendix Ⅲ： Serum（Blood Plasma）AST Assay

1. Pretreatments of blood serum (or plasma) assay samples: Take samples and start assay directly.

（If enzyme activity is higher than 150 units, then dilute with physiological saline and assay again.）

2. Operation table：

	Assay well	Contrast well
Matrix solution（μl）already pre-warmed at 37°C	20	20
Sample to assay（μl）	5	

When add sample to each assay well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 30 minutes.

2,4-dinitro-phenylhydrazine solution	20	20

（μl）		
Sample to assay（μl）		5

When add sample to each contrast well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37℃ water bath or air bath for 20 minutes.

0.4mol/L NaOH solution（μl）	200	200

Shake 96-well plate softly and horizontally to mix well, place at room temperature for 15 minutes, use ELIASA to measure OD values of wells at 510 nm, according to $OD_{Absolute} = OD_{Assay} – OD_{Contrast}$, check standard curve to acquire corresponding AST/GOT activity units.

3. Example：

Take 5μl SD rat blood serum, operate on 96-well plate according to table above, use ELIASA to measure OD values at 510nm, in results, OD_{Assay} is 0.2853, $OD_{Contrast}$ is 0.2128, $OD_{Absolute} = OD_{Assay} – OD_{Contrast}$ = 0.0725, substitute in fitting formula acquired by standard curve: Blood serum AST activity = 24.4210 Carmen's unit = 11.7709U/L.