GENLISA™ Alanine Aminotransferase (ALT/GPT) Assay

REF : KBCA1051

Ver 1.1

RUO

Biochemical Assay for theQuantitative Determination of Alanine Aminotransferase (ALT/GPT) in serum, plasma tissue cells, cell culture supernatants and other biological samples.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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REF KBCA1051 Σ **96 tests**

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Introduction:

The GENLISA™ Assay kits are used for assessing the specific biomarker in samples analytes which may be serum, plasma, tissue cells and cell culture supernatant as validated with the kit. The kit employs in vitro quantitative determination of Alanine Aminotransferase in the sample.

Intended Use:

The GENLISA™ Alanine Aminotransferase (ALT/GPT) Assay kit is used as an analytical tool for quantitative determination of Alanine Aminotransferase (ALT/GPT) in serum, plasma, tissue cells, cell culture supernatants and other biological samples.

Principle:

At condition of 37ºC and PH7.4, alanine transarninase（ALT）affects on substrate composed by alanine and α-oxoglutarat, produces pyruvic acid andb glutamic acid. After react for 30 minutes (fixation time), add 2,4-dinitro-phenylhydrazine (DNPH) hydrochloric acid solution in order to terminate reaction and produce pyruvic phenylhydrazone by addition reaction of DHPH and carbonyl in ketonic acid. Phenylhydrazone appears red brown at alkaline condition, so it is able to measure absorbances at 505nm to calculate enzyme activity.

Materials Provided:

1. Reagent 1 (Alanine transarninase matrix solution) – 1 vial x 5 ml
2. Reagent 2 (2,4-dinitro-phenylhydrazine solution) – 1 vial x 5 ml
3. Reagent 3 (4 mol/l NaOH solution) – 1 vial x 5 ml
4. Reagent 4 (2 umol/ml sodium pyruvate standard solution) – 1 vial
5. Reagent 5 (0.1mol/l phosphate buffer) – 1 vial
6. Microwell Plate (96 wells) - 1 no.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Clean tubes and Eppendorf tubes
6. Precision single and multi-channel pipette and disposable tips.

Handling/Storage:

1. All reagents should be stored as indicated on the component label and keep away from the light
2. Standard should be stored as indicated on the vial label.
3. All the reagents should be used within 12 months from manufacturing date.
4. Before using, bring all components to room temperature (18-25ºC). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

Reagent Preparation

1. Alanine transarninase matrix solution: 5 ml x 1 vial, can be stocked at 4ºC in fridge for 6 months;
2. 2,4-dinitro-phenylhydrazine solution:5 ml x1 vial can be stocked at 4ºC for 6 months;
3. 4 mol/l NaOH solution: 5 ml x 1 vial, can be stocked hermetically at room temperature for 6 months; dilute this solution with distilled water at ratio of 1:9 to make 0.4 mol/l NaOH solution before use.
4. 2 umol/ml sodium pyruvate standard solution: 1 vial can be stored at 4ºC for 6 months.
5. 0.1 mol/l phosphate buffer: 1 vial, can be can be stored at 4ºC for 6 months.

Assay Procedure

	Assay Wells	Standard Wells
Matrix solution (ul) already pre -warmed at 37ºC	20	20
Sample to assay (ul)	5	
After pipetting the matrix solution and samples, mix well. Incubate the plate in 37ºC water bath or air bath for 30 minutes.		
2,4-dinitro-phenylhydrazine solution (ul)	20	20
Sample to assay (ul)		5
When add sample to each Standard well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37ºC water bath or air bath for 20 minutes.		
0.4mol/L NaOH solution (ul)	200	200

Shake the plate softly and horizontally to mix well.
Incubate at room temperature for 15 minutes.
Use ELISA to measure the OD values of the wells at OD510nm.
$OD_{Absolute} = OD_{Assay} – OD_{Standard}$.
Interpolate from the standard curve to aquire the corresponding ALT/GPT activity units.

Note

1. 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 Standarding 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 minute at 25ºC oxidates NADH to NAD+, absorbance decreasing caused by this oxidation per 0.001 is considered as 1 unit (1 Carmen's unit = 0.482 IU/L, 25ºC).
2. Generally, the amount of endogenous ketonic acid in serum sample is very low, so serum Standard 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 Standard tube for every sample, you can use reagent blank tube instead, but it needs to make Standard tube for every sample of heavy lipidemia, jaundice and haemolysis.
3. If enzyme activity is higher than 150 units, then dilute sample with physiological saline and assay again.

4. You should consider absorbance in common serum Standard well (or named as sample blank well) as one of quality daily control index; if there is big difference, then some reasons such asα-oxoglutarate, DHPH concentration and instruments should be considered.
5. ALT in blood serum can be stocked at room temperature (25ºC) for 2 days, at 0～ 4ºC for 1 week, at 25ºC for 1 month.

Appendix 1: ALT Standard Curve

Standard Curve Preparation：

	0	1	2	3	4	5
0.1 mol/l Phosphate Buffer (ul)	5	5	5	5	5	5
2 umol/ml Sodium Pyruvate Standard Solution (ul)	0	2	4	6	8	10
Matrix Buffer (ul)	20	18	16	14	12	10
2,4-Dinitro-Phenylhydrazine Solution (ul)	20	20	20	20	20	20

Mix the plate well. Incubate in 37^{0}C water bath or air bath for 20 minutes.

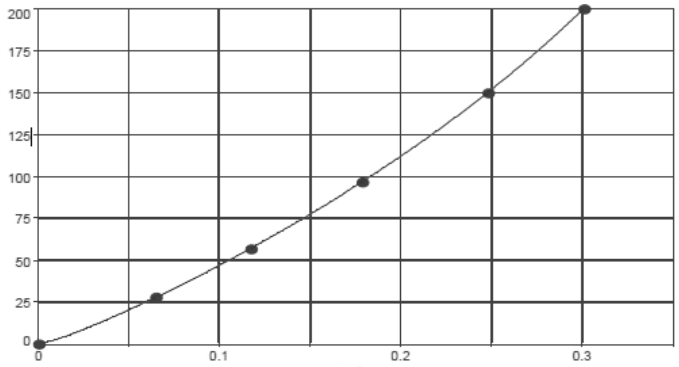
0.4mol/L NaOH solution (ul)	200	200	200	200	200	200

Shake the plate softly and horizontally to mix well.
Incubate at room temperature for 15 minutes.
Use ELISA to measure the OD values of the wells at OD510nm.
$OD_{Absolute} = OD_{Assay} – OD_{Standard}$.
Interpolate using Carmen;s unit as ordinates to aquire the corresponding ALT/GPT activity units.

Appendix: Referenced Standard Curve:

OD values Institute experiments	0.2169	0.2819	0.3340	0.3957	0.4651	0.5179
$OD_{Absolute}$ values Institute	0	0.0650	0.1171	0.1788	0.2482	0.3010
Corresponding enzyme activities in Carmen's unt	**0**	**28**	**57**	**97**	**150**	**200**

Typical Graph



Appendix II: Tissue ALT Assay

1. Sample Pretreatment

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 **(ALT content in liver tissue is relatively high, so generally it need to dilute with physiological saline to 1% homogenate to assay).**

2. Assay Procedure

	0	1	2	3	4	5
0.1 mol/l Phosphate Buffer (ul)	5	5	5	5	5	5
2 umol/ml Sodium Pyruvate Standard Solution (ul)	0	2	4	6	8	10
Matrix Buffer (ul)	20	18	16	14	12	10
2,4-Dinitro-Phenylhydrazine Solution (ul)	20	20	20	20	20	20

Mix the plate well. Incubate in 37^{0}C water bath or air bath for 20 minutes.

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

Shake the plate softly and horizontally to mix well.
Incubate at room temperature for 15 minutes.
Interpolate the Tissue ALT values as per th formaula indicated below.

Tissue ALT activity (U/gm protein) = ALT Activity acquired by Standard Curve ÷ Protein Concentration in homogenate to assay

Note: gm protein / L = gram protein per Ltr

Example:
Take a piece of liver tissue of *Acipenser sinensis*, make 10% homogenate at mass-weight ratio of 1:9, dilute homogenate with physiological saline to 0.5% , take 5μl sample and assay as per table above.
Use an ELISA reader to measure absorbances at 510nm.
In results,
ODAssay is 0.3041,
ODStandard is 0.2242,
protein concentration in 0.5% liver homogenate of *Acipenser sinensis* is 0.3311 gm prot/L,
ODAbsolute = ODAssay – ODStandard = 0.079, substitute in fitting formula acquired by standard curve, calculate as follows:
ALT activity in liver tissue of *Acipenser sinensis* (U/gm prot) = 35.8979 Carmen's unit x 0.482 ÷ 0.3311 = 52.2585 (U/gprot)

Appendix III Serum (Blood Plasma) ALT Assay

1. Sample Pretreatment

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

2. Assay Procedure

	Assay Wells	Standard Wells
Matrix solution (ul) already pre -warmed at 37ºC	20	20
Sample to assay (ul)	5	
After pipetting the matrix solution and samples, mix well. Incubate the plate in 37ºC water bath or air bath for 30 minutes.		
2,4-dinitro-phenylhydrazine solution (ul)	20	20
Sample to assay (ul)		5
When add sample to each Standard well, please insert tip to matrix solution at bottom of well, blow and mix repeatedly. After this step, place plate in 37ºC water bath or air bath for 20 minutes.		
0.4mol/L NaOH solution (ul)	200	200

Shake the plate softly and horizontally to mix well.
Incubate at room temperature for 15 minutes.
Use ELISA to measure the OD values of the wells at OD510nm.
ODAbsolute = ODAssay – ODStandard.
Interpolate from the standard curve to aquire the corresponding ALT/GPT activity units.

Example:

Take 5 ul human blood serum, assay according to the table above.
Use an ELISA reader to measure OD values at 510nm.
In results,
ODAssay is 0.2541,
ODStandard is 0.2215,
ODAbsolute = ODAssay – ODStandard = 0.0326, substitute in fitting formula acquired by standard curve: Blood serum ALT activity = 12.0121 Carmen's unit = 5.7898 U/l.

Quality Control:

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

Performance Characteristics of the Kit:

Negative Blank
Blank tube absorbance value should be ≤0.200.

Assay Concentration Range
Concentration range is 0-9.04 mM with r^2 >0.995.
Note: The absorbance value A is around 0.2200-0.2900 for the 2.26mM Standard solution.

Precision
Inter and Intra Assay ≤ 10%

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

LIMITED WARRANTY

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