α1-Antitrypsin Assay Kit (AAT)

Method:Immunoturbidimetric Method

INTENDED USE

For in vitro quantitative determination of α1-antitrypsin in human serum or plasma.

CLINICAL SIGNIFICANCE

α1-antitrypsin (α1-antitrypsin, AAT) is synthesized mainly in the liver and has a molecular weight of 54 kD. Among the proteins capable of inhibiting proteases in serum and plasma, the amount and activity of α1-antitrypsin occupy an important position. It specifically inactivates serine proteases (such as trypsin, chymotrypsin, urokinase, collagenase, elastase, plasmin, and thrombin, etc.) [1], and can form reversible enzyme inhibition complexes with these proteases, in order to Maintain the integrity of tissue cells. α1-antitrypsin is an important acute phase response protein in inflammatory reactions (such as infections and rheumatoid diseases), tissue necrosis, and increased concentrations during trauma [1]. Hepatic parenchymal cell inflammation is often accompanied by elevated antitrypsin levels, while other acute-phase reactants remain unaffected. Defective α1-antitrypsin gene results in a deficiency of hereditary antitrypsin, can not inhibit protease activity, and is likely to cause tissue cell damage [2]; at the same time, abnormal α1-antitrypsin accumulates in liver cells and causes liver damage [1]. The onset was neonatal, fetal respiratory distress syndrome, hepatitis, and cirrhosis [3-5]. Cirrhosis occurs most often in adults with alpha1-antitrypsin deficiency [1, 3, 4]. In addition, proteolytic enzymes do not have α1-antitrypsin inhibition, and they excessively affect the elastic fibers of the alveolar wall, leading to emphysema in early years (20-30 years) [2].

ASSAY PRINCIPLE

The α1-antitrypsin in the sample reacts with the anti-α1-antitrypsin antibody to form an insoluble immune complex, which makes the reaction solution appear turbid. The turbidity is positively correlated with the α1-antitrypsin content in the sample.

REAGENT COMPOSITION

Contents	Concentration of Solutions
Reagent 1 (R1)	
Phosphate buffer Sodium chloride Polyethylene glycol	20.0 mmol/L 150.0 mmol/L ＞3.0% w/v
Reagent 2 (R2)	
Phosphate buffer Sodium chloride α1-antitrypsin antiserum	20.0 mmol/L 150.0 mmol/ ＞30.0%

STORAGE AND EXPIRY DATE

1. The reagent is sealed and stored in dark at 2-8 ℃ for 12 months.
2. Reagents should not be frozen.

SAMPLE REQUIREMENT

Fresh serum.

EDTA sodium salt, heparin lithium anticoagulant plasma..

ASSAY METHOD

Test Conditions

Main wavelength	600 nm	Sample（S）	2 μl
Secondary wavelength	800 nm	Reagent 1（R1）	150μl
Reaction temperature	37℃	Reagent 2（R2）	50μl
Cuvette diameter	1cm	Reaction type	End-point method

3. Operate procedure

Add into colorimetric cup：	
Sample（S）	2μl
Reagent 1（R1）	150μl
Mix well and incubate for 5 minutes at 37 ℃, read initial absorbance A1;	
Reagent 2（R2）	50μl
Mix well and incubate for 5 minutes at 37 ℃, read final absorbance A2; Calculate △A=A2-A1	

Note: Parameters above are only introduced with Hitachi 917 as an example. The parameters of different biochemistry analyzers are slightly different. Please read

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the manual carefully before setting parameters. For more detail parameters, please call technical engineers from the BSBE.

4. Calibration procedure

Gcell AAT calibrator is recommended to use.

Calibrator traces to the international reference materials ERM-DA470k/IFCC.

4.1 According to the requirements of the calibration procedure in the operation manual of biochemistry analyzer, each laboratory establishes its own calibration procedure according to the specific conditions.

4.2 Requirements for calibration and frequency: It is recommended to calibrate at least once every two weeks. When the following situations occur, it is recommended to re-calibrate: change the reagent batch number, the indoor quality control runs out of control, the biochemistry analyzer carries out major maintenance or replaces the main parts such as light source or cuvette.

5. Control procedure

Choose control matching the BSBE's calibrator. The absorbance of quality control should be within the labeled value range. If the results deviate from the scope, please find out the reason by following steps:

5.1 Check the parameter setting and light source.

5.2 Check the cleanliness of the cuvette and sampling needle.

5.3 Check whether water is contaminated or not. Bacterial growth can lead to incorrect results.

5.4 Check the reaction temperature.

5.5 Check the validity of the kit.

6. Result calculation

Setting calibration curve by calibrator concentrations against the corresponding ΔA values. The concentration of AAT in the sample is obtained by ΔA value read from the calibration curve.

REFERENCE RANGE

The results of 120 serum samples and 120 plasma samples from "normal persons" in Beijing area show that:

Normally serum or plasma: 90-200 mg/dL.

Laboratories are suggested to establish its own reference interval according to age, sex, diet and region.

PRODUCT PERFORMANCE CHARACTERISTICS

ACCURACY

The kit was tested with ERM-DA470k/IFCC international reference material. The deviation of the results should not exceed (±10%).

SENSITIVITY

When the sample concentration is 150.0mg/dL, the change of absorbance should not less than 0.1000.

LINEARITY

In the [30.0, 460.0] mg/dL interval, the linearity correlation coefficient r ≥ 0.990. In the [30.0, 60.0] mg/dL interval, the linearity deviation measured should not exceed ±6.0 mg/dL, In the [60.0, 460.0] mg/dL interval, the linearity deviation measured should not exceed±10%.

PRECISION

Repeatability

The coefficient of variation (CV%) of the measured values should be no more than 10% when serum samples or quality control of different concentrations (High/low) are repeated measured for 10 times.

Batch difference

The relative range (R) of three randomly choosen kits should be no more than 10%.

INTERFERENCES

The effect of bilirubin ≤ 600 mg/dL, hemoglobin ≤ 1000 mg/dL, RF ≤ 1000IU//mL, TG ≤ 800mg/dLwas less than 10%.

MATTERS FOR ATTENTION

1. The reagent contains preservatives. If it enters the eyes, mouth or contact on the skin, please rinse it thoroughly with clean water immediately and go to the hospital if necessary.

2. The reagent contains preservatives, which can react strongly with copper, lead and other metals to form azide metal. Therefore, please dilute the waste liquid and flush the drain pipe to avoid residual when disposal.

3. Do not mix or exchange reagents with different batches in the process of detection.

4. Opened reagents should be sealed and stored according to the specified method. Expired product should not be

used.

5. Please dispose test tubes and other instruments that have touched the test sample according to the relevant medical waste disposal regulations.

6. Calibrator and control use human matrix serum, passed the detection of HIV (HIV 1, HIV 2) antibodies, hepatitis B surface antigen (HbsAg) and hepatitis C virus (HCV). All of them are negative. Although the detection method is highly accurate, it can not be guaranteed that all infected donors are found, so the control should also be treated as infectious specimens.

REFERENCES

1.Janciauskiene SM，Bals R，Koczulla R，et al．The discovery of α1-antitrypsin and its role in health and disease［J］．Respir Med，2011，105(8)：1129-1139

2.Sun Z，Yang P．Role of imbalance between neutrophil elastase and alpha 1-antitrypsin in cancer development and progression［J］．Lancet Oncol，2004，5(3)：182-190

3.Fregonese L，Stolk J．Hereditary alpha-1-antitrypsin deficiency and its clinical consequences［J］．Orphanet J Rare Dis，2008，19；3：16．

4.Vidal R，Blanco I，Casas F，et al．Guidelines for the diagnosis and management of alpha-1 antitrypsin deficiency［J］．Arch Bronconeumol，2006，42(12)：645-659

5.Johnson AM，Alper CA．Deficiency of α1-antitrypsin in childhood liver disease．Pediatrics 1970；46(6)：921-925

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