PRODUCT INFORMATION & MANUAL

Total Bile Acid/TBA Assay Kit (Colorimetric)

NBP3-25822

For research use only.
Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

8th Edition, revised in February, 2018

（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Total Bile Acid (TBA) Colorimetric Assay Kit

Catalog No: NBP3-25822
Method: Colorimetric method
Specification: 100Assays (Can detect 100 samples with spectrophotometer or 400 samples with biochemical analyzer and microplate reader without duplication)
Measuring instrument: Spectrophotometer, Biochemistry analyzer, Microplate reader
Detection range: 0-180 μmol/L

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

The kit is used for the quantitative determination of the total bile acid concentration in serum.

Detection significance

Total bile acid (TBA) is mainly used for the screening and prognosis of follow-up of hepatobiliary disease and as the marker of liver parenchymal damage and cholestasis. The increase of TBA indicates the risk of viral hepatitis, cirrhosis, alcoholic liver disease, drug-induced liver injury or cholestasis.

Detection principle

$$\text{S-NAD} + \text{Bile acid} \xrightarrow{3\alpha-\text{HSD}} \text{3-Ketosteroid} + \text{S-NADH}$$

$$\text{3-Ketosteroid} + \text{S-NADH} \xrightarrow{\text{Diaphorase}} \text{NAD} + \text{Bile acid}$$

Measure the OD value at 405 nm and the changes of absorbance is proportional to the concentration of bile acid.

Kit composition

Reagent	Ingredient	Content	Size	Storage
Reagent 1	Glycine buffer	pH 4.0	75 mL ×1 vial	2-8℃, 12 months
	S-NAD	1 g/L		
	Stabilizer	8.2 mg/L		
Reagent 2	Bile acidase (3α-HSD)	≥ 30 KU/L	25 mL ×1 vial	2-8℃, 12 months (shading light)
	NADH	≥ 1.5 mmol/L		
Standard	Sodium glycocholate	50 μmol/L	1 mL ×1 vial	

Sample requirements

1. Separate serum within 2 hours after blood collection. The serum sample can be stored at 15~30℃ within 8 hours, at 2~8℃ for a week or at -20℃ for 3 months.
2. Interfering substances: conjugated bilirubin ≤ 5mg/dL, unconjugated bilirubin ≤ 20mg/dL, vitamin C ≤ 1mg/dL, triglyceride ≤ 9.25 mmol/L, hemoglobin ≤ 100mg/dL have no effect to the results.

Operation steps

1. Detection with spectrophotometer

	Blank tube	Standard tube	Sample tube
Double distilled water (μL)	10		
Standard (μL)		10	
Sample (μL)			10
Reagent 1 (μL)	720	720	720
Mix fully and incubate at 37℃ for 5 min.			
Reagent 2 (μL)	240	240	240
Mix fully and incubate at 37℃ for 1 min. Set spectrophotometer to zero with double distilled water and measure the absorbance at 405 nm at 0 second (A1) and 3 min (A2), respectively. Calculate the ΔA=A2-A1.			

2. Detection with microplate reader

	Blank tube	Standard well	Sample well
Double distilled water (μL)	2.5		
Standard (μL)		2.5	
Sample (μL)			2.5
Reagent 1 (μL)	180	180	180
Mix fully and incubate at 37℃ for 5 min.			
Reagent 2 (μL)	60	60	60
Mix fully and incubate at 37℃ for 1 min. Measure the absorbance at 405 nm at 0 second (A1) and 3 min (A2), respectively. Calculate the ΔA=A2-A1.			

3. Detection with biochemical analyzer

Setting parameter

Temperature	37℃	Method	Two-point end point method
Dominant wavelength	405 nm	Optical path	1 cm
Reaction direction	Up	Sample	2.5 μL
Reagent 1	180 μL	Reagent 2	60 μL
Incubation time (Sample+ Reagent 1)	5 min		
Incubation time (Sample+ Reagent 1+ Reagent 2)	1 min		

Measure the absorbance at 0 second (A1) and 180 second (A2), respectively. Calculate the ΔA=A2-A1.

Automatic biochemical analyzer has its own program parameter input language. Reagents matches the analyzer and carry out automatic measurement after the above basic parameters are modified.

Calculation of results

TBA content ($\mu mol/L$) = $\frac{\triangle A_{Sample}-\triangle A_{Blank}}{\triangle A_{Standard}-\triangle A_{Blank}}$ × Concentration of standard ($\mu mol/L$)

Reference range

1.2~10.5 μmol/L (This data is for reference only. It is recommended that each laboratory establish its own range of reference values.)

Technical parameter

1. **Linear range**: 0-180 μmol/L, $r^2 \geq 0.990$.
2. **Accuracy**: inaccuracy ≤ 15.0%.
3. **Recovery rate:** 100 ±20%
4. **Precision**: intra-CV ≤ 5.0%, inter-CV ≤ 10.0%.
5. **Absorbance for the blank control (reagents only) ≤ 0.7** (405 nm wavelength, 1 cm optical path).

Notes

1. Instructions should be followed strictly, changes of operation may result in unreliable results.
2. The validity of kit is 12 months. Do not freeze the kit. The standard can be stably stored at 2~8℃ for 1 month after opening.
3. Do not use components from different batches of kit.
4. The sample needs to be diluted with normal saline before the determination when the concentration of TBA is higher than 180 μmol/L. The result should be multiplied by the dilution factor.
5. The kit is for research use only and contains preservatives. It should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
6. The ratio of sample and reagent can be scaled as required.
7. The reaction time can be prolong to 5 min or 10 min from 3 min if the ∆A is less than 0.003.