Catalog #80460

V.4

Crystal Chem

Rat Total Bile Acids Kit Instructions

For the quantitative determination of total bile acids
in rat serum and plasma

Catalog #80460
96 Assays

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The Rat Total Bile Acids kit is for the quantitative determination of total bile acids in rat serum and plasma. The total bile acids concentration is expressed as µmol/L. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Total bile acids is an important test for monitoring normal liver function. Total bile acids are metabolized in the liver and are present in increased concentrations with abnormal liver function.

C. Principle of the Assay

The Rat Total Bile Acids kit is based on enzymatic technology that utilizes the unique enzymatic property of 3-a hydroxy-steroid dehydrogenase. In the presence of NAD, the bile acids are converted into 3-keto steroids and NADH. The NADH formed reacts with nitrotetrazolium blue (NBT) to form a dye. The dye formation is monitored by measuring absorbance at 540nm and is directly proportional to the bile acids concentration in the rat sample.

D. Kit Storage

1. Upon receipt of the Rat Total Bile Acids kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
CC1	Reagent CC1 (Liquid)	1 X 20 mL
CC2	Reagent CC2 (Liquid)	1 X 4 mL
CC3	Reagent CC3 (Powder)	2 X 10 mL
CAL1	Calibrator 1 (Liquid)	1 X 2 mL

E.2. Materials required but not provided

Microplates
Micropipettes and disposable tips
Clean glass tubes and test tube racks
Volumetric flasks
Incubator (37ºC)
Distilled water
Microplate reader or spectrophotometer or analyzer (should read A_{540} values)
0.9% saline

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human & bovine sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Reagents CC1 and CC2 contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.
4. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (20 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Fresh serum or EDTA treated plasma samples should be used. Hemolysed or heparinized samples should not be used.

Note: *Samples should be collected under fasting conditions as total bile acid concentrations increase after meals. It is recommended that samples be used within 1 week of collection when stored refrigerated. If assay is to be performed more than 1 week after collection, samples should be frozen.*

I. Assay Procedure

I.1. Preparation of reagents

Prior to running the assay, Reagent CC3 must be reconstituted using Reagent CC1. Add 10 mL of Reagent CC1 to one bottle of Reagent CC3 and dissolve by swirling gently. Reconstituted Reagent CC3 is stable for 1 week at 2-8°C.

All reagents should be stored at 2-8°C immediately after use. Do not freeze reagents, calibrators, or controls. Before use, bring all reagents to room temperature and mix the reagents thoroughly by gentle agitation or swirling.

I.2. Preparation of samples, calibrators, and controls

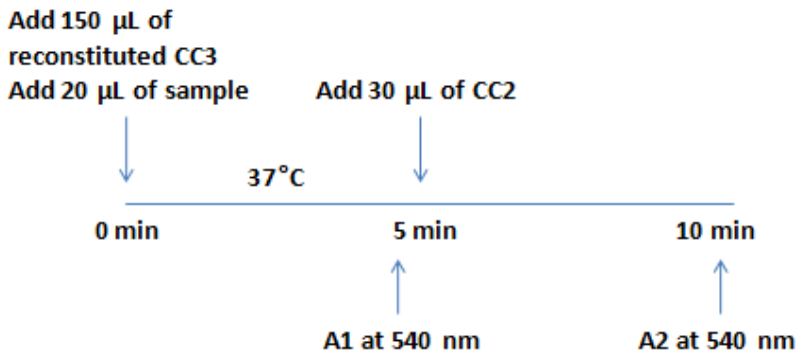
1. Bring all samples, calibrators, and controls to room temperature. Frozen samples should be allowed to fully thaw before proceeding.
   Note*: In addition to running the calibrator provided, the assay requires running a blank calibrator. Distilled water should be used for running the blank calibrator. Optional controls are sold separately (Cat# 80463). Controls should be reconstituted with 1 mL of* H_2O.

I.3. Assay procedure

The procedure below reflects a manual procedure performed using a microplate and a microplate reader (ideal when running multiple samples manually). The procedure can be easily adopted as needed to be run in a glass tube with a spectrophotometer. The assay can also be adopted to work on various automated analyzers using the schema presented in Figure 1. Please contact Crystal Chem for instrument-specific information.

1. Add 150 µL of reconstituted Reagent CC3 and 20 µL of sample, calibrator, or control into each well (as needed) of a microplate and mix well by repeated pipetting.
2. Place microplate in incubator (37ºC) and allow microplate to equilibrate to 37ºC over 5 minutes.
3. Zero the absorbance of plate reader at 540nm.
4. Measure absorbance using a plate reader (measure A_{540} values).
   Note: The Rat Total Bile Acids assay is an end-point assay and the first reading point A1 is right before the addition of reagent CC2.
5. Add 30 µL of Reagent CC2 and mix well by repeated pipetting.
6. Measure the increase in absorbance after 5 minutes at 37ºC using a plate reader (measure A_{540} values).

Figure 1. Summary of assay procedure



I.4. Determining the rat total bile acids concentration

1. Calculate the change in absorbance ΔA (0sec ~ 300sec) for each well.

   $\Delta A = (OD_{540nm,\ 300sec}) - (OD_{540nm,\ 0sec})$

2. Using linear graph paper, construct the total bile acids calibration curve by plotting the mean change in absorbance value for the calibrator (incl. blank) on the Y axis versus the corresponding total bile acids concentration on the X axis. ***Note:*** *Calibrator value varies per lot and should be obtained from the calibrator label. The calibrator and blank should be run every time the assay is performed.*

3. Rat total bile acid concentrations in the samples are interpolated using the calibration curve and mean change in absorbance values for each sample. The total bile acids concentration is expressed as µmol/L. This interpolation can be simplified using Equation 1 below.
   Note: *Samples with a high total bile acids concentration (180 µmol/L or higher) should be diluted with the 0.9% saline and rerun.*

Equation 1. Calculation of rat total bile acids concentration

Rat BA concentration =

[(sample ∆A540 – blank ∆A540) / (calibrator ∆A540 – blank ∆A540)] × calibrator conc.

J. Performance characteristics

J.1. Assay range

The Rat Total Bile Acids assay has a linear range from 0-180 µmol/L.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

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