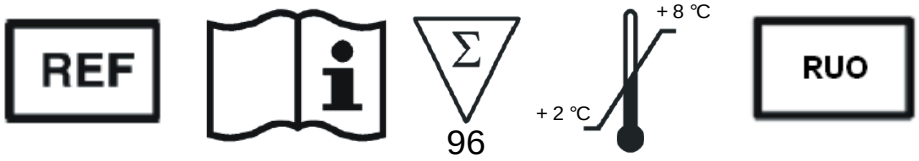
Manual

Bile acids

Colorimetric test system for the determination of bile acids in stool

Valid from 28.10.2022



IC7500

ImmuChrom GmbH
Lise-Meitner-Str. 13
D 64646 Heppenheim
Tel.: ++49 6252 910084
Fax: ++ 49 6252 910070
Info@immuchrom.de
www.immuchrom.de

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1. Intended use

This photometric test is suitable for the quantitative determination of free bile acids in stool. For *research* use only.

2. Introduction

Bile acids play a significant role in the absorption of lipids and fat-soluble vitamins in the small intestine, as well as in the excretion of cholesterol. Furthermore, they contribute to the stimulation of intestinal motility.

Bile acids serve, among other things, the digestion of fats, are formed in the liver and released into the duodenum with the bile. In the last section of the small intestine, about 95-97% of the bile acids are reabsorbed (enterohepatic circulation).

If the absorption of bile acids in the small intestine is impaired, they are increasingly excreted in the stool, which leads to irritation of the colon and causes severe diarrhea, resulting in severe fluid loss, vitamin and mineral deficiencies. This is called bile acid malabsorption syndrome or chologenic diarrhea.

Indications

- Diarrhea
- Steatorrhea

The ImmuChrom bile acids kit allows an easy, rapid and precise quantitative determination of bile acids in stool samples. The kit includes all reagents ready to use for preparation of the samples.

3. General notes, warnings and precautions

All reagents of this kit are strictly intended for research use only.

Individual components from different batches and test kits should not be interchanged. The expiry dates stated on the relevant packaging must be observed.

The test kit reagents contain preservatives to protect against bacterial growth. Therefore contact with the skin and/or mucous membranes should be avoided.

Avoid contact of the stop solution, which consists of acid, with the skin. It causes burns on contact. You should therefore work with protective gloves and goggles. In the event of contact, the burned area must be immediately and thoroughly rinsed with plenty of water. If necessary, a doctor should be consulted.

Adherence to the prescribed protocol for performing the test is essential. ImmuChrom GmbH assumes no liability for any damage caused by unauthorized changes in the test procedure.

The guidelines for carrying out quality control in medical laboratories must be observed. Appropriate controls must be carried along.

The reagents must not be used after the expiration date.

Wear disposable gloves when handling specimens or kit reagents and wash hands thoroughly afterwards. Do not pipette by mouth. Do not eat, drink, smoke, or put on make-up in areas where specimens or kit reagents are being handled.

Samples may contain unknown interfering substances. This can lead to false high or false low results.

4. Material delivered in the test package

Article no.	Component	Description	Amount
IC7500mtp	MTP	Microtiter plate	2 x 12 x 8 wells
IC7510st	STD 0-4	Standards (1 ml)	5 vials
IC7510ko	CTRL 1-2	Control 1 and 2 (2 ml)	2 vials
IC7400ex	EXT	Extraction buffer	150 ml
IC7510su	SUB	Substrate	2 x 20 ml
IC7510el	Enzym	Enzyme solution	10 ml
IC7510sp	STOPP	Stop solution	55 ml

5. Additional special equipment

- Centrifuge, 3000xg
- Plastic vials
- Stool sample extraction vials
- Various pipettes
- Multichannel- or multipipette
- Foil to cover the microtiter plate
- Microtiter plate reader with filter 405 nm (reference filter 620 nm)
- Vortex mixer

6. Reagent preparation

Microtiter plate (MTP). Take the needed number of strips and assemble them on the holder. Stripes which are not needed yet can be stored at 20-25 °C. Please do not dispose of the holder until all stripes are used.

All other test reagents are stable at 2-8 °C, up to the date of expiry stated on the label. Slowly bring the reagents to room temperature before use.

7. Specimen

Stool samples

The bile acids are extracted by the extraction buffer out of the stool sample.

Extraction in stool extraction vials

In a stool sample extraction vial mix 15 mg stool with 1.5 ml EXT, then vortex it until the mixture is homogenous. Transfer the resulting slurry to a plastic vial and centrifuge it for 10 min at 3000xg.

The supernatant is directly pipetted into the microtiter plate wells with no further dilution.

8. Procedure

Principle of the method

The quantitative determination of bile acids is carried out via their enzymatic oxidation to 3-keto steroids with the formation of thio-NADH. The resulting change in absorbance (ΔOD) at 405 nm is determined photometrically. The bile acids concentration can be calculated from the standard curve.

Sample preparation

All reagents and samples should be warmed up to 20-25 °C and mixed well before use.

The position of standards, controls and samples are noted on a protocol sheet.

Important:

To ensure the reproducibility of the measurement, the given incubation times and temperature should be followed strictly.

1. Pick out the pre-assembled microtiter plate with the needed number of stripes. Pipette **20 µl STD**, **CTRL** or supernatant of the **samples** in horizontal double values into the microtiter plate.

2. Add **200 µl** SUB to every cavity containing STD, CTRL or sample.

3. Incubate for **5 min** at room temperature (20-25 °C) in the dark.

4. Add **50 µl** enzyme as quickly as possible and without interruptions vertically to every second strip (e.g. strip 1, 3, 5, 7, 9, 11).

   Caution: The reaction starts immediately after enzyme addition. Interruptions or a too long period of enzyme addition can lead to incorrect or non-evaluable measurement results.

5. Incubate for **7 min** at room temperature (20-25 °C) in the dark.

   Caution: Exceeding the incubation time can lead to incorrect or non-evaluable measurement results.

6. Add **50 µl** STOPP as quickly as possible and without interruptions vertically to every second strip (e.g. strip 1, 3, 5, 7, 9, 11).

7. **Measurement:** The **absorbance** is measured immediately at **405 nm** in the microtiter plate photometer with reference wavelength 620 nm. Absorbance values of cavities **without** enzyme and STOPP are subtracted from the corresponding cavities **with** enzyme and STOPP (**ΔOD**).

9. Calculation of analytical results

For calculating the results, a “point to point” curve is recommended.

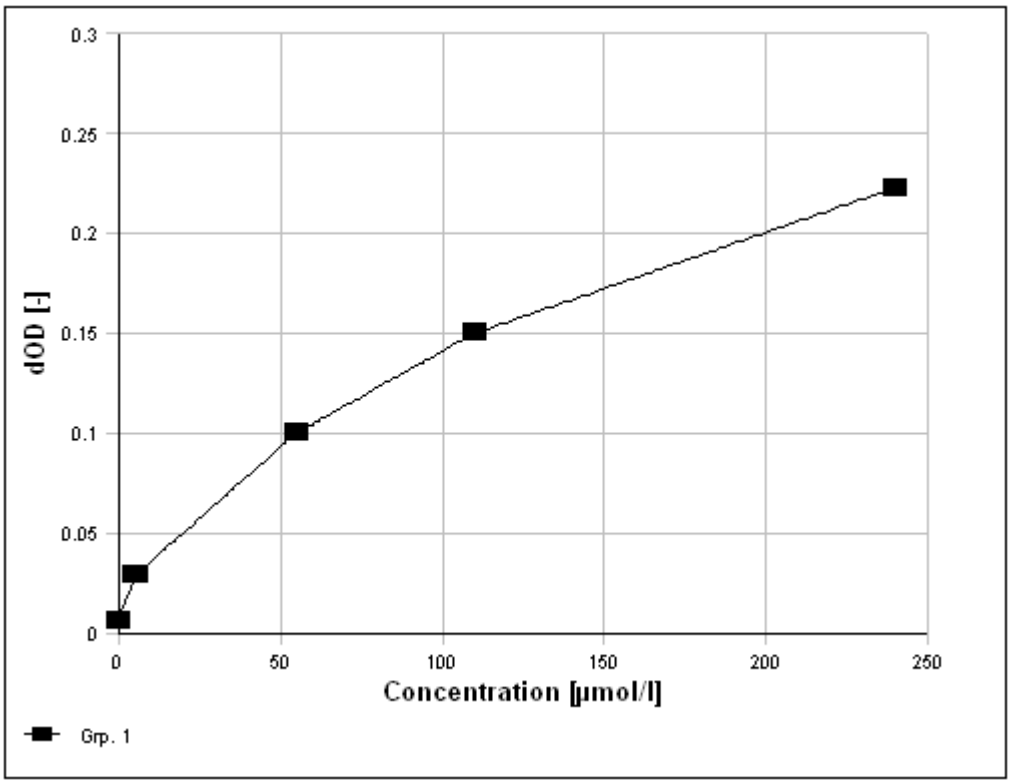
Stool samples

15 mg stool in 1.5 ml extraction buffer corresponds to a dilution of 1:100.

The determined bile acid concentration must be multiplied by the dilution factor **100**.

When using different dilutions, multiply the measured sample concentrations by the corresponding factor.

Standard curve



The curve given above is only for demonstration. It must not be used for calculation of your samples.

10. Internal quality control

Reference values

Stool: 0.53 – 7.01 µmol/g or 530 – 7010 µmol/l

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

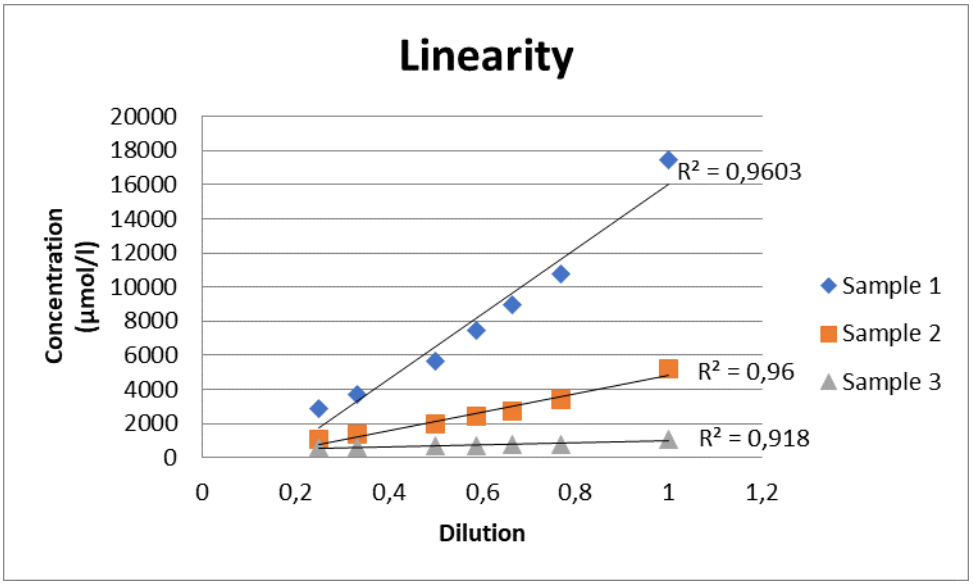
11. Validation data

Precision and reproducibility

Intra-Assay CV:	2.5 % (508 µmol/l)	[n = 10]
	4.7 % (2248 µmol/l)	[n = 10]
	10.7 % (6263 µmol/l)	[n = 10]
Inter-Assay CV:	7.6 % (466 µmol/l)	[n = 10]
	9.9 % (1787 µmol/l)	[n = 10]
	13.6 % (6281 µmol/l)	[n = 10]

Linearity

Sample	Dilution factor	Expected [µmol/l]	Measured [µmol/l]	Recovery [%]
1	--	--	17480	
	1:1.3	13539	10760	79.5
	1:1.5	11787	8983	76.2
	1:1.7	10448	7466	71.5
	1:2	8941	5625	62.9
	1:3	6095	3683	60.4
	1:4	4671	2838	60.8
2	--	--	5235	
	1:1.3	4120	3358	81.5
	1:1.5	3624	2709	74.8
	1:1.7	3245	2410	74.3
	1:2	2818	1961	69.6
	1:3	2013	1353	67.2
	1:4	1610	1063	66.0
3	--	--	1043	
	1:1.3	895	783	87.5
	1:1.5	829	754	90.9
	1:1.7	779	700	89.8
	1:2	723	675	93.5
	1:3	616	605	98.3
	1:4	562	577	103



Detection limit

1.91 µmol/l

For the determination, the zero-standard was measured 12 times. The 3-fold standard deviation was added to the mean value. The respective concentration was read from the standard curve.

Recovery

Sample	Endogenous [µmol/l]	Added [µmol/l]	Expected [µmol/l]	Measured [µmol/l]	Recovery [%]
1	744	734 3670 7341	1478 4415 8085	1282 4619 7208	86,7 105 89.2
2	2223	1043 5215 10430	3266 7438 12653	3027 6528 11460	92.7 87.8 90.6
3	6175	1043 5215 10430	7218 11390 16605	6591 10800 15730	91.3 94.8 94.7

12. Limitations of the method

Stool samples with concentrations above the standard curve, are diluted with extraction buffer and determined again.

Decreased levels may be measured in cases of severe diarrhea.

13. Disposal

The substrate (SUB) must be disposed as non-halogenated solvent.

14. Literature references

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