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Manual

IDK® Bile Acids

For the in vitro determination of bile acids in stool

Valid from 2020-05-12

REF **K 7878W** Σ 96

REF **K 7878W.20** Σ 20 x 96

+8 °C
+2 °C

IVD

CE

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1. INTENDED USE

This photometric assay is intended for the quantitative determination of bile acids in stool. For *in vitro* diagnostic use only.

2. INTRODUCTION

Bile acids are produced in the liver as end-products of cholesterol metabolism. Together with other components of the liver bile, such as cholesterol, bilirubin, phospholipids and proteins, bile acids are secreted into the duodenum.

Important functions of bile acids are the excretion of cholesterol, absorption of fatty acids and fat-soluble vitamins in the small intestine as well as stimulation of intestinal motility.

The majority of the secreted bile acids are reabsorbed in the terminal ileum and returned to the liver via the portal venous system for eventual recirculation in a process known as enterohepatic circulation; only a small proportion (3–5 %) are excreted into the feces.

If the enterohepatic recycling of bile acids fails, excess amounts of bile acids enter the colon and are lost with the feces; this condition is called bile acid malabsorption.

Indications

Suspected bile acid malabsorption

- After resection of the terminal ileum
- Crohn's Disease affecting the terminal ileum
- Radiation enteritis
- Post-cholecystectomy
- Post-vagotomy Celiac disease
- Chronic pancreatitis
- Idiopathic bile acid malabsorption

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 7878W	K 7878W.20
K 7878W	PLATE	Microtiter plate	12 x 8 wells	20 x 12 x 8 wells
K 7878W	STD	Standards, ready-to-use (0; 6; 12; 24; 48; 96 µmol/l)	1 x 6 vials	20 x 6 vials

Cat. No.	Label	Kit components	Quantity for cat. no.	
			K 7878W	K 7878W.20
K 7878W	CTRL1	Control, ready-to-use (see specification for range)	1 x 1 vial	20 x 1 vial
K 7878W	CTRL2	Control, ready-to-use (see specification for range)	1 x 1 vial	20 x 1 vial
K 6999.C.100	IDK Extract®	Extraction buffer concentrate *IDK Extract*® 2.5x	1 x 100 ml	10 x 100 ml
K 7878W	RGZ1	Reagent 1, ready-to-use	1 x 20 ml	20 x 20 ml
K 7878W	RGZ2	Reagent 2, ready-to-use	1 x 6 ml	20 x 6 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Stool sample application system such as Cat. No.: K 6998SAS
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.**
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.

- **Preparation of the extraction buffer:** The **extraction buffer concentrate *IDK Extract*®** has to be diluted with ultra pure water **1:2.5** before use (100 ml *IDK Extract*® + 150 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at 37 °C in a water bath. The ***IDK Extract*®** is stable at **2–8 °C** until the expiry date stated on the label. Extraction buffer (1:2.5 diluted *IDK Extract*®) can be stored in a closed flask at **2–8 °C for 4 months**.
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Raw stool can be stored for 3 days at room temperature, 7 days at 2–8 °C or 2 years at -20 °C. Avoid more than two freeze-thaw cycles.

Stool extracts (1:100) can be stored for 3 days at room temperature, 7 days at 2–8°C or 14 days at -20 °C. Avoid more than three freeze-thaw cycles.

Extraction of the stool samples

Extraction buffer (1:2.5 diluted *IDK Extract*®) is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 1.5 ml extraction buffer:

Applied amount of stool:	15 mg
Buffer Volume:	1.5 ml
Dilution Factor:	1:100

Please follow the instructions for the preparation of stool samples using the SAS as follows:

a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Fill the **empty stool sample tube** with **1.5 ml extraction buffer** (1:2.5 diluted *IDK Extract*®) before using it with the sample. **Important:** Allow the extraction buffer to reach room temperature.

c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

d) Vortex the tube well until no stool sample remains in the notches. **Important:** Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with buffer for ~ 10 minutes improves the result.

e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.

f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution : **1:100**

For analysis, pipet **10 µl of this dilution** per well.

7. ASSAY PROCEDURE

Principle of the test

This assay is designed for the quantitative determination of bile acids in stool.

In the presence of excess thio-NAD, bile acids are converted to 3-keto steroids by the enzyme 3-α-hydroxysteroid dehydrogenase while thio-NADH is formed.The rate of formation of thio-NADH can be determined by the change of absorbance (ΔOD) at 405 nm.

A dose response curve ΔOD vs. concentration is generated, using the values obtained from measured standards. The bile acids concentration of the samples is determined directly from this curve.

Test procedure

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips covered at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	Add each **10 µl standards/controls/diluted samples** into the respective wells.
2.	Add **150 µl reagent 1** (RGZ1) with a repeater pipet into each well. Take care not to contaminate the dispenser tip with standards/controls/samples.
3.	Incubate the strips for **5 min** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Add **50 µl reagent 2** (RGZ2) with a repeater pipet into each well. Take care not to contaminate the dispenser tip with the content of the well.
5.	Incubate the strips for **1 min** at room temperature (15–30 °C) on a **horizontal shaker***.
6.	Determine **absorption immediately** with an ELISA reader at **405 nm** against 620 nm (or 690 nm) as a reference. If the photometer allows the measurement of reaction kinetics, record 10 measuring points in a time interval of 15 sec and determine the slope (**ΔOD)** by linear regression over all data points between 15 and 150 sec.. If only single measurement is possible, then determine absorption directly after the 1-minute-incubation, cover the plate for 2 min (please note the exact time interval) and then take a second measurement. The slope (**ΔOD)** corresponds to the difference of final OD and start OD divided by the time interval between the two measurements. ΔOD = (final OD - start OD)/time interval

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

8. RESULTS

Point-to-point calculation

We recommend a linear ordinate for the change in optical density (**ΔOD)** and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Stool

The obtained results have to be multiplied by the **dilution factor of 100** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations of bile acids above the highest standard can be further diluted with sample extraction buffer and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

LoB × *sample dilution factor to be used*

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik AG studies of stool samples of apparently healthy persons (n = 1 179), the following values were estimated (1 g stool ≙ 1 ml):

90 % reference range	0.78–6,54 µmol/g	(≙ 780–6 540 µmol/l))
Median	2.79 µmol/g	(≙ 2 790 µmol/l)

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 40

The repeatability was assessed with 2 stool samples under **constant** parameters (same operator, instrument, day and kit lot).

Sample	Mean value [µmol/l]	CV [%]
1	8.13	3.4
2	25.86	5.2

Reproducibility (Inter-Assay); n = 22

The reproducibility was assessed with 2 stool-samples under **varying** parameters (different operators, instruments, days and kit lots).

Sample	Mean value [µmol/l]	CV [%]
1	8.46	5.4
2	27.04	5.9

Analytical sensitivity

The following values have been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB	0.344 µmol/l
Limit of detection, LoD	1.894 µmol/l
Limit of quantitation, LoQ	1.894 µmol/l

The evaluation was performed according to the CLSI guideline EP17-A2. The specified accuracy goal for the LoQ was 20 % CV.

Accuracy – Trueness

The trueness states the closeness of the agreement between the result of a measurement and the true value of the measurand. Therefore, bile acids spikes with known concentrations were added to 2 different stool samples. The results below were obtained without consideration of the sample dilution factor.

Sample [µmol/l]	Spike [µmol/l]	Expected [µmol/l]	Obtained [µmol/l]	Recovery [%]
8.26	4.57	12.44	13.20	106.17
	8.73	16.24	15.19	93.53
	12.52	19.70	18.13	92.01
25.01	4.57	28.39	29.60	104.27
	8.73	31.46	32.76	104.12
	12.52	34.27	36.38	106.16

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A with a serial dilution of 2 different stool samples.

For bile acids in stool in stool, the method has been demonstrated to be linear from 1.89 to 54.83 µmol/l based on the standard curve without considering possibly used sample dilution factors, showing a non-linear behaviour of less than ± 20 % in this interval.

Sample	Dilution	Expected [µmol/l]	Obtained [µmol/l]	Recovery [%]
A-1	1:100	52.10	52.10	100.00
	1:200	26.05	22.28	85.51
	1:400	13.03	10.29	78.98
	1:800	6.51	4.86	74.63
	1:1600	3.26	2.56	78.50

Sample	Dilution	Expected [µmol/l]	Obtained [µmol/l]	Recovery [%]
A-2	1:100	54.82	54.82	100.00
	1:200	27.41	26.65	97.21
	1:400	13.71	12.74	92.96
	1:800	6.85	6.54	95.44
	1:1600	3.43	3.00	87.49
	1:3200	1.71	1.98	115.41
B-1	1:200	39.05	39.05	100.00
	1:400	19.53	20.79	106.48
	1:800	9.76	10.36	106.13
	1:1600	4.88	4.84	99.07
	1:3200	2.44	2.82	115.43
B-2	1:200	42.44	42.44	100.00
	1:400	21.22	21.11	99.49
	1:800	10.61	10.72	101.07
	1:1600	5.31	5.39	101.56
	1:3200	2.65	3.44	129.85

12. PRECAUTIONS

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Avoid contact with skin or mucous membranes.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.

- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- *IDK*® and *IDK Extract*® are trademarks of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

1. Camilleri, M., 2014. Advances in understanding of bile acid diarrhea. *Expert review of gastroenterology & hepatology*, **8**(1), pp.49–61.
2. Halilbasic, E., Claudel, T. & Trauner, M., 2013. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *Journal of Hepatology*, **58**(1), pp.155–168.
3. Vijayvargiya, P. et al., 2013. Methods for diagnosis of bile acid malabsorption in clinical practice. *Clinical Gastroenterology and Hepatology*, **11**(10), pp.1232–1239.
4. Müller-Lissner, S. a & Pirk, O., 2002. Irritable bowel syndrome in Germany. A cost of illness study. *European journal of gastroenterology & hepatology*, **14**(12), pp.1325–1329.

Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by


Attention


Consult instructions for use

Consult specification data sheet