BILE ACIDS (TBA)

ENZYMATIC COLORIMETRIC
MANUAL

INTENDED USE

For the quantitative *in vitro* determination of Bile Acids in serum and plasma. This product is suitable for Manual use.

Cat. No.

BI 2672	R1a. Blank Reagent	10 x 10 ml
10 x 10 ml	R1b. Reconstituting Buffer	1 x 105 ml
	R2. 3α-HSDH	1 x 30 ml

GTIN: 05055273200713

ASSAY PRINCIPLE

$$\text{3}\alpha\text{-hydroxy bile acids} + NAD^+ \xrightarrow{\text{3}\alpha\text{-HSDH}} \text{3-keto-hydroxy bile acids} + NADH + H^+$$

$$NADH + H^+ + NBT \xrightarrow[\Delta^4\text{DH}]{\text{Diaphorase}} NAD^+ + \text{formazan}$$

3α-hydroxy bile acids are converted to the corresponding 3-keto bile acids in the presence of NAD^+ by 3α-hydroxysteroid dehydrogenase (3α-HSDH). The NADH formed reacts with nitrotetrazolium blue (NBT) in a Δ^4DH and Diaphorase catalyzed reaction to form a formazan dye, which has a stable blue colour with an absorption maximum at 546 nm. The intensity of the colour produced is directly proportional to the bile acids concentration in the sample.

SAMPLE

Serum, plasma.

REAGENT COMPOSITION

Contents	Initial Concentration of Solutions
R1a. Blank Reagent	
Diaphorase	250 U/l
NAD^+	2 mmol/l
Nitrotetrazolium blue (NBT)	0.25 mmol/l
Oxamic Acid	45 mmol/l
3 - Oxo - 5β - steroid Δ^4 – dehydrogenase	0.4K U/l
R1b. Reconstituting Buffer	
Sodium phosphate	65 mmol/l, pH 7.0
EDTA	0.77 mmol/l
R2. 3α-HSDH	
3α-hydroxysteroid dehydrogenase	≥ 500 U/l

SAFETY PRECAUTIONS AND WARNINGS

For *in vitro* diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Solution R1b contains Sodium Azide. Avoid ingestion or contact with skin or mucous membranes. In case of skin contact, flush affected area with copious amounts of water. In case of contact with eyes or if ingested, seek immediate medical attention.

Sodium Azide reacts with lead and copper plumbing, to form potentially explosive azides. When disposing of such reagents, flush with large volumes of water to prevent azide build up. Exposed metal surfaces should be cleaned with 10% sodium hydroxide.

Health and Safety Data Sheets are available on request.

Please dispose of all biological and chemical materials according to local guidelines.

The reagents must be used only for the purpose intended by suitably qualified laboratory personnel, under appropriate laboratory conditions.

STABILITY AND PREPARATION OF REAGENTS

R1a. Blank Reagent
Reconstitute the contents of one bottle of Reagent R1a with **10 ml** of Buffer R1b. Stable for 1 week at +2 to +8°C or 24 hours at +20°C.

R1b. Reconstituting Buffer
Contents ready for use. Stable up to the expiry date when stored at +2 to +8°C.

R2. 3α-HSDH
Contents ready for use. Stable up to expiry date when stored at +2 to +8°C.

MATERIALS PROVIDED

Reconstituting Buffer
3α-HSDH
Blank Reagent

MATERIALS REQUIRED BUT NOT PROVIDED

Randox Assayed Multi-sera Level 2 (Cat. No. HN 1530) and Level 3 (Cat. No. HE 1532)
Randox Calibration Serum Level 3 (Cat. No. CAL 2351)

PROCEDURE NOTES

A Reagent blank may be performed by replacing sample or standard with redistilled water.

PROCEDURE 1 REAGENT START

Wavelength:	546 nm
Cuvette:	1 cm light path
Temperature:	37°C
Measurement:	against water

Pipette in cuvette:

	Sample Blank	Sample	Calibrator Blank	Calibrator
Sample	200 µl	200 µl	--	--
Calibrator	--	--	200 µl	200 µl
Blank Reagent (R1)	400 µl	400 µl	400 µl	400 µl

Mix, incubate for 2 to 5 minutes at 37°C.

3α-HSDH (R2)	--	100 µl	--	100 µl
Deionised Water	100 µl	--	100 µl	--

Mix, incubate for exactly 5 minutes at 37°C, and read the absorbance of the sample (A_{sample}), sample blank ($A_{sample\ blank}$), calibrator($A_{calibrator}$) and calibrator blank ($A_{calibrator\ blank}$) immediately.

REAGENT PREPARATION FOR PROCEDURE 2 WITH SAMPLE START

Prepare working reagent by adding 1 ml of 3α-HSDH to 4 mls of reconstituted blank reagent. The blank reagent is prepared by adding 1 ml of deionised water to 4 mls of the reconstituted blank reagent. Stable for 1 week at +2 to +8°C or 24 hours at +20°C.

PROCEDURE 2

Wavelength:	546 nm
Cuvette:	1 cm light path
Temperature:	25°C, 37°C
Measurement:	against water

Pipette in cuvette:

	Sample Blank	Sample	Calibrator Blank	Calibrator
Sample	200 µl	200 µl	--	--
Calibrator	--	--	200 µl	200 µl
3α-HSDH (R2)	--	500 µl	--	500 µl
Blank Reagent (R1)	500 µl	--	500 µl	--

Mix, incubate for exactly 20 minutes at +25°C or 15 minutes at 37°C. Read the absorbance of the Sample (A_{sample}), Sample Blank ($A_{sample\ blank}$), Calibrator ($A_{calibrator}$) and Calibrator Blank ($A_{calibrator\ blank}$) immediately.

CALCULATION

1. Calculate the net absorbance value for the Calibrator and sample as follows:

$$\Delta A_{calibrator} = A_{calibrator} - A_{calibrator\ blank}$$

$$\Delta A_{sample} = A_{sample} - A_{sample\ blank}$$

The bile acid concentration of the sample can be calculated using the net absorbance in the following equation:

$$\text{concentration} = \frac{\Delta A\ \text{sample}}{\Delta A\ \text{calibrator}} \times \text{concentration of calibrator}$$

CALIBRATION

We recommend that this assay should be calibrated using Randox Calibration Serum Level 3.

QUALITY CONTROL

Randox Assayed Multi-sera, Level 2 and Level 3 are recommended for daily quality control. Two levels of controls should be assayed at least once a day. Values obtained should fall within a specified range. If these values fall outside the range and repetition excludes error, the following steps should be taken:

1. Check instrument settings and light source.
2. Check cleanliness of all equipment in use.
3. Check water, contaminants i.e. bacterial growth may contribute to inaccurate results.
4. Check reaction temperature.
5. Check expiry date of kit and contents.
6. Contact Randox Laboratories Customer Technical Services, Northern Ireland +44 (0) 28 94451070.

INTERFERENCE

Avoid heparinized plasma as this will interfere with the assay. Extreme lipaemic conditions may be a further source of interference.
Haemoglobin was tested up to 50 mg/dl and found not to interfere.

NORMAL RANGE [3]

Human Serum (fasting): 0 - 6 µmol/l

It is recommended that each laboratory establish its own reference range to reflect the age, sex, diet and geographical location of the population.

LINEARITY

The method is linear up to a concentration of 150 µmol/l. Samples above this concentration should be diluted (up to four times) with 0.9% saline. (0.15 mol/l NaCl).

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