fortress diagnostics

ISO 13485 accredited company

BXC0432A
2x60ml
STORE AT 2 – 8°C
FOR IN-VITRO DIAGNOSTIC USE ONLY

LDL CHOLESTEROL

Enzymatic (Colorimetric)

Kit Contents:

		BXC0432A
R1	LDL Precipitant	2 x 60 ml
R4	Cholesterol Standard	1 x 5 ml

Test Principle:
Low density lipoproteins are precipitated by the addition of heparin at their isoelectric point (pH 5.04). The HDL and VLDL remain in the supernatant and can be determined by enzymatic methods.

LDL Cholesterol = Total Cholesterol – Cholesterol in the supernatant

Reagent Concentration:

LDL Precipitant R1	Heparin	50,000 IU/l
	Sodium Citrate pH 5.04	0.064 mmol/l
Cholesterol Standard R4		1.29 mmol/l (50 mg/dl)

Reagent Handling and Preparation:
R1: LDL Precipitant:
The Precipitant reagent R1 is supplied ready to use and will be stable stored at the recommended temperature until the expiry date quoted.
R4: Cholesterol Standard:
Supplied ready to use and is stable up to the expiry date when stored at 2-8°C.
R2: Reagent required for measurement of Cholesterol:
Cholesterol CHOD-PAP Cat. No BXC0261A or BXC0261B

Sample:
Serum

Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
500 nm (Hg 546nm)	20-25°C, 37°C	1 cm light path	Against reagent blank

Precipitation step:
Mix 100 µl of sample with 1000 µl precipitating reagent and keep at room temperature for 10 minutes and then centrifuge at 4000 rpm for 15 minutes. The cholesterol concentration of the supernatant can be determined within 1 hour after centrifugation. Pipette into test tubes as follows:

	Reagent Blank	Standard	Sample
Distilled Water	50 ul	—	—
Standard	—	50 ul	—
Supernatant	—	—	50 ul
Reagent R2	1 ml	1 ml	1 ml

Mix and incubate for 10 minutes at room temperature or for 5 minutes at 37°C then measure the absorbance of the samples and standard against the reagent blank.
The endpoint will be stable for 1 hour.

Calculation:
Using a Standard:
Concentration of Cholesterol in the supernatant =

$$\frac{\text{Abs. sample}}{\text{Abs. standard}} \times \text{conc of standard}$$

Using a factor:
Concentration of Cholesterol in the supernatant =
Sample Absorbance x Factor as follows:

	mmol/l	mg/dl
Hg 546 nm	49.63	1920
500 nm	32.70	1265

The LDL cholesterol result is calculated using the following formula:

LDL Cholesterol = Total Cholesterol - Cholesterol in the supernatant

For Calibration we recommend Fortress aqueous LDL-Cholesterol Standard (1.29mmol/l (50 mg/dl)). If this standard is used, then the results must be multiplied by 11 to allow for the dilution effect of the precipitation step on serum.
N.B. The precipitation step should not be carried out on aqueous standards.

Linearity:
The method will be linear up to 20 mmol/l (774 mg/dl)

Expected Values:

	Mg/dl	Mmol/l
Optimal	< 100	<2.59
Near or above borderline	100-129	2.59 - 3.35
Borderline high	130 - 159	3.36 -4.12
High	160-189	4.13 - 4.89
Very high	>190	>4.90

These values are supplied as a guideline only.
It is recommended that a laboratory establishes its' own reference range, as this can be influenced by many factors. National Cholesterol Education Program (NCEP) Guidelines
LDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex.

Use on Automated Analysers:
The cholesterol determination is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.

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HDL CHOLESTEROL

PRECIPITANT

Kit Contents:

	BXC0422A
R1 HDL Precipitant R4 Cholesterol Standard	2x60ml 1x5ml

Test Principle:
Low density lipoproteins are precipitated by the addition of phosphotungstic acid in the presence of magnesium ions.
The HDL fraction remains in the supernatant and this is determined by cholesterol assay.

Reagent Concentration:

Precipitant R1	Phosphotungstic Acid	0.55mmol/l
	Magnesium Chloride	25mmol/l
Cholesterol Standard R4	Magnesium Chloride	1.29mmol/l (50mg/dl)

Reagent Handling and Preparation:
For Macro assays, The reagents are supplied ready to use and will be stable up until the expiry date when stored at room temperature (20-25°C).
For Semi-micro assays, Pre-dilute the precipitating reagent in 4+1 ratio with distilled water i.e. to one of 60ml bottles of HDL precipitating reagent, add 15ml distilled water. This is stable up until the expiry date when stored at room temperature (20-25°C).

R2 Additional Reagents required for Cholesterol CHOD-PAP assay: BXC0261A

Sample:
Serum, Heparinised plasma or EDTA plasma

Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
500nm (Hg 546nm)	20-25°C, or 37°C	1cm light path	Against Reagent Blank

Precipitation Procedure:

Pipette into test tubes as follows:		
	Macro	Semi Macro
Sample	500µl	200µl
HDL Precipitant R1	1000µl	-
Diluted Precipitant	-	500µl
Mix and incubate for 10 minutes at room temperature. Then centrifuge for 10 minutes at 4000 rpm. Remove the clear supernatant within 2 hours and determine the cholesterol content by the CHOD-PAP method. The supernatant may be stored up to 5 days at 2-25°C.		

Cholesterol CHOD-PAP Procedure:

Only one reagent blank per series. Pipette into test tubes:			
	Reagent Blank	Standard	Sample
Distilled water	100µl	-	-
Supernatant	-	-	100µl
Standard R4	-	100µl	-
Reagent R2	1ml	1ml	1ml
Mix and incubate for 10 minutes at room temperature or 5 minutes at 37°C. Measure the absorbance of the sample and standard against the reagent blank within 60 minutes.			

Calculation:
HDL Cholesterol:
When using a factor

	Macro		Semi-Micro	
Wavelength	Mmol/l	Mg/dl	Mmol/l	Mg/dl
500nm	4.65	180	5.43	210
Hg 546nm	7.09	274	8.27	320

When using a Standard

$$\text{HDL Conc in the supernatant} = \frac{\text{Abs. sample}}{\text{Abs. standard}} \times \text{conc of standard}$$

For calibration Fortress HDL cholesterol standard is only used to calibrate the cholesterol assay and should not be diluted in the precipitation step. The standard value must be multiplied by 3 if using the macro method and by 3.5 if using the semi micro method to compensate for the dilution effect on serum of the precipitation step.

LDL-Cholesterol

$$\text{LDL-Cholesterol in mmol/l} = \text{total cholesterol} - \frac{\text{triglycerides}}{2.2} - \text{HDL-Cholesterol}$$

$$\text{LDL-Cholesterol in mg/dl} = \text{total cholesterol} - \frac{\text{triglycerides}}{5.0} - \text{HDL-Cholesterol}$$

Linearity:
The method will be linear up to 20mmol/l (774 mg/dl)

Interferences:
The assay is unaffected by:
Icteric samples (bilirubin < 30mg/dl), rheumatoid factors < 1000IU/mL

Haemolytic samples
(Hb < 500mg/dl) and lipaemic samples (triglyceride < 1200mg/dl).

Lipaemic samples
With a triglyceride concentration >1200 mg/dl should be diluted 1 + 9 with 0.9% (w/v) NaCl before assay. The corresponding result should be multiplied by 10.

As HDL cholesterol is affected by a number of factors such as smoking, exercise, hormones, age and sex, each laboratory should establish its own reference ranges.

Expected Values: (NCEP GUIDELINES)

	Low	High
mg/dl	<40	≥60
	<1.04	≥1.55

Use on Automated Analysers:
The cholesterol determination is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.

Quality Control
It is recommended that a laboratory uses normal and abnormal reference sera to verify the performance of any procedure. Results should fall within the specified ranges for the controls.

Fortress Lipid Control Cat No BXCO330A/316A

If results fall outside the acceptable range appropriate action as determined by the laboratory's internal quality procedures should be taken.

Health & Safety:
This kit is designed for use by suitably qualified laboratory personnel only. Exercise the normal precautions required for the handling of laboratory reagents. Do not ingest the material. Dispose of material according to local guidelines.

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BXC0261A	BXC0261B	BXC0261C	BXC0261D
2x60ml	6x60ml	8x250ml	4x250ml
STORE AT 2-8°C			
FOR IN-VITRO DIAGNOSTIC USE ONLY			

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CHOLESTEROL

CHOD-PAP (LIQUID STABLE)

Kit Contents:		**BXC0261A**	**BXC0261B**	**BXC0261C**
R1	Reagent	2x60ml	6x60ml	8x250ml
R4	Standard	1x5ml	1x5ml	1x5ml

		BXC0261D
R1	Reagent	4x250ml
R4	Standard	1x5ml

Test Principle:
Cholesterol is present in serum as cholesterol esters and free cholesterol. The Cholesterol esters present in serum are hydrolysed by cholesterol esterase and the cholesterol is then measured by oxidizing with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide in turn reacts with phenol and 4-aminoantipyrine present to form the red quinoneimine dye. The intensity of the dye formed is directly proportional to the level of cholesterol present in the sample.

Clinical Significance:
Elevated levels of cholesterol are primarily considered as an indication of increased risk of cardiovascular disease and should be taken into consideration combined with the overall lipid profile.

Reagent Concentration:

	Pipes Buffer	50mmol/l
	Cholesterol Oxidase	>100U
	Cholesterol Esterase	>150U
	4-aminoantipyrine	0.3mmol/l
	Peroxidase)	>800U
	Phenol	6.0mmol/l
Standard	Cholesterol	200mg/dl

Reagent Handling and Preparation:
The reagent is supplied ready to use. It is stable for up to the expiry date when kept at 2-8°C and protected from light. The reagent may develop a slight pink colouration. This will not affect performance providing the OD remains <0.300 when measured against a water blank at 505nm.

Sample:
Serum/Heparinised Plasma
EDTA plasma may also be used but tends to give lower results.

Specimen:
Do not use citrate, oxalate or fluoride.
Fasting and nonfasting samples can be used.
Centrifuge samples containing precipitate before performing assay

Stability:	5-7 days at +2 to +8°C
	3 months at -20°C

Manual Procedure:

Wavelength	**Temperature**	**Cuvette**	**Measurement**
500 nm, Hg 546nm	20-25°C, 37°C	1cm light path	Against Reagent Blank

Pipette into test tubes as follows:		
	Reagent Blank	**Calibrator/Sample**
DDH_2O	10µl	-
Calibrator/smpl	-	10µl
Reagent (R1)	1000µl	1000µl
A serum based calibrator or an aqueous cholesterol standard can be used to calibrate this assay. Mix well and incubate for 10 minutes at 20-25°C or 5 minutes at 37°C, then Read the absorbance of the sample or standard against the reagent blank. The endpoint is stable for 60 minutes.		

Calculation:
Cholesterol conc in sample = $\frac{\Delta abs\ Sample}{\Delta abs\ Standard}$ x conc., of standard

Using a Factor:
500nm 21.7 x ΔA (840mg/dl x ΔA)
Hg 546 nm 14.3 x ΔA (553mg/dl x ΔA)

Linearity:
The test is linear up to a cholesterol concentration of 20mmol/l (774mg/dl). Dilute samples above this concentration with 0.9% NaCl and reassay, multiplying the result by the dilution factor.

Sensitivity:
Cholesterol levels of 0.20mmol/l (7.74mg/dl) can be measured accurately by this method.

Precision:

Cholesterol conc (mmol/l)	**Intra Assay - Within Run**	
	n	**CV%**
4.50	20	2.07%
5.18	20	1.69%

Cholesterol conc (mmol/l)	**Inter Assay - Between Run**	
	n	**CV%**
4.50	10	2.9%
5.18	10	2.2%

These characteristics were determined using an AU600 analyser. Results will vary depending on the system in use.

Limitations - Interference:
Haemoglobin values up to 200mg/dl & bilirubin values up to 5mg/dl do not interfere with the test.

Normal Values:

Cholesterol Level	**Clinical Interpretation**
< 5.2mmol/l (200mg/dl)	Normal
5.2-6.2mmol/l (200-239mg/dl)	Borderline Risk
> 6.2mmol/l (240mg/dl)	High Cholesterol
These values are intended only as a guideline. Cholesterol levels can vary seasonally, according to geographic location and with time of sampling. At least 2 measurements should be made on separate occasions and the results should be taken in conjunction with other clinical and laboratory information.	

Use on Automated Analysers:
This reagent is suitable for use on a range of automated analysers. Specific instructions for these applications are available on request from our technical department.
For automated use we recommend a serum based calibrator to eliminate any matrix bias which may be observed with the aqueous standard.

Fortress Calibrator Cat No BXC0321K/L/M

Quality Control:
It is recommended that a laboratory uses normal and abnormal reference control sera to verify the performance of the procedure, both performance of the reagent and any instrumentation employed in the determination. Results obtained should fall within the specified ranges.

Fortress Normal Bovine Assayed Control Cat No BCX0313A (10x5ml)
Fortress Elevated Bovine Assayed Control Cat No BCX0313B (10x5ml)

Fortress Normal Human Assayed Control Cat No BXC0312A (10x5ml)
Fortress Elevated Human Assayed Control Cat No BXC0312B (10x5ml)

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BXC0271A	BXC0271B	BXC0271C	BXC0271D
2x60ml	4x60ml	5x20ml	12x60ml
STORE AT 2-8°C			
FOR IN-VITRO DIAGNOSTIC USE ONLY			

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TRIGLYCERIDES

GPO-PAP MONOLIQUID

Kit Contents:	BXC0271A	BXC0271B	BXC0271C	BXC0271D
R1 Buffer	2x60ml	4x60ml	5x20ml	12x60ml
R4 Standard	1x5ml	1x5ml	1x5ml	1x5ml

Intended Use:
Enzymatic in vitro test for the quantitative determination of triglycerides in human serum and plasma.

Test Principle:
Enzymatic colorimetric test:

$$\text{Triglycerides} + 3H_2O \xrightarrow{LPL} \text{Glycerol} + 3RCOOH$$

$$\text{glycerol} + ATP \xrightarrow{GK,\ Mg2+} \text{G-3-P} + ADP$$

$$\text{G-3-P} + O_2 \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{p-chlorophenol} \xrightarrow{POD} \text{4-(p-benzoquinone-monoimino)-phenazone} + 2H_2O + HCl$$

Clinical Significance:
Triglycerides are esters of the trihydric alcohol glycerol with 3 long-chain fatty acids. They are partly synthesized in the liver and partly ingested in food.
The determination of triglycerides is utilized in the diagnosis and treatment of patients having diabetes mellitus, nephrosis, liver obstruction, lipid metabolism disorders and numerous other endocrine diseases.

Reagent Concentration:

Buffer 1	Pipes Buffer pH 7.8	50mmol/l
	p-Chlorophenole	2mmol/l
	Lipoprotein Lipase	150000U/l
	Glycerolkinase	800U/l
	Glycerol-3-P-Oxidase	4000U/l
	Peroxidase	440U/l
	4-Aminoantipyrine	0.7mmol/l
	ATP	0.3mmol/l
	Mg2+	40mmol/l
	Na-cholate	0.20mmol/l
	Potassium-Hexacyanoferrate (II)	1µmol/l
Standard 4	Triglycerides Concentration of 200mg/dl	2.24mmol/l

Reagent Handling and Preparation:
Buffer 1: Ready for use.
Standard 4: Ready for use.
Unopened kit components: Up to the expiry date at +2°C to +8°C.
Stability 3 weeks at +20 to +25°C.
Colouration of the reagent (reagent blank at 546nm, 1cm >0,2) indicates a contamination or damage by storage at higher temperatures.

Sample:
Collect serum using standard sampling tubes.
Heparinized or EDTA plasma.
Centrifuge samples containing precipitate before performing the assay.

Stability	5-7 days at +2°C to +8°C
	3 months at -20°C

Testing Procedure:
Materials Provided:
- Working Solutions as described above

Additional Materials Required:
- Controls as indicated below
- 0.9% NaCl

Manual Procedure:

Wavelength	Temperature	Cuvette	Measurement
546nm (500-550nm)	+37°C	1cm light path	Against Reagent Blank / one Reagent Blank per series only

Pipette into test tubes as follows:		
	Reagent Blank	Samples/Standards
Sample/Calibrator	-	10µl
Working Reagent	1000µl	1000µl
Mix, measure after incubating at +37°C for 5min or at +20 to +25°C for 10min. Within 60mins read absorbance of sample against reagent blank.		

Calculation:

$$\frac{\Delta abs\ sample}{\Delta abs\ standard} \times \text{Standard conc.} = \text{Triglycerides conc.}$$

Linearity:
Measuring /reportable range:
3-1000 mg/dl (0.05-11.4mmol/l)
Determine samples with higher concentrations via the rerun function. On instruments without rerun function, manually dilute samples with 0.9% NaCl (e.g. 1+4). Multiply the result by the appropriate dilution factor (e.g. 5)

Sensitivity:
Detection limit : 3mg/dl (0.09mmol/l)
The lower detection limit represents the lowest measurable triglycerides concentration that can be distinguished from zero.

Imprecision:
Reproducibility was determined using human samples the following results were obtained:

Intra Assay - Within Run			
Sample	Mean mg/dl	SD mg/dl	CV%
Control Serum 1	122	1.09	0.89
Control Serum 2	150	1.79	1.19
Control Serum 3	206	1.44	0.70

Inter Assay - Between Run			
Sample	Mean mg/dl	SD mg/dl	CV%
Control Serum 1	121	1.96	1.62
Control Serum 2	161	1.84	1.14
Control Serum 3	204	2.36	1.16

Normal Values:

According to the recommendations of the European Arteriosclerosis Society. Lipid Metabolism Disorder.		
Cholesterol Triglycerides	< 200mg/dl (2.30mmol/l)	No
Cholesterol	200-300mg/dl	Yes if HDL-CHOL <35mg/dl
Cholesterol Triglycerides	>300mg/dl >200mg/dl	Yes

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes the triglycerides results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.
Note: If the free glycerol is to be taken into account, then 10mg/dl (0.1 mmol/l) must be subtracted from the triglycerides value obtained. For control sera, note the target value declaration of the manufacturer.

Method comparison:
A comparison of the Fortress Triglycerides Monoliquid (y) with a commercial obtainable assay (x) gave the following result:
y = 1.002 x + 1.136; r = 0.999

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