BECKMAN COULTER

AU/DxC AU US

Instructions For Use

TRIG
TRIGLYCERIDE

REF OSR60118 4 x 20 mL R1, 4 x 5 mL R2
OSR61118 4 x 50 mL R1, 4 x 12.5 mL R2
OSR66118 4 x 173 mL R1, 4 x 48 mL R2

For *in vitro* diagnostic use only.

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of Triglyceride concentrations in human serum and plasma on Beckman Coulter AU/DxC AU analyzers.

SUMMARY AND EXPLANATION

Triglycerides are the major form of fat found in nature and their primary function is to provide energy for the cell.[1] Measurements of triglyceride are used in the diagnosis and treatment of patients with diabetes mellitus, nephrosis, liver obstruction, other diseases involving lipid metabolism, or various endocrine disorders.[2]

Clinically, triglyceride assays are used to help classify the various genetic and metabolic lipoprotein disorders and in the assessment of risk factors for atherosclerosis and coronary artery disease.[3,4]

METHODOLOGY

This Triglyceride procedure is based on a series of coupled enzymatic reactions.[5,6] The triglycerides in the sample are hydrolyzed by a combination of microbial lipases to give glycerol and fatty acids. The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to produce glycerol-3-phosphate. The glycerol-3-phosphate is oxidized by molecular oxygen in the presence of GPO (glycerol phosphate oxidase) to produce hydrogen peroxide (H_2O_2) and dihydroxyacetone phosphate. The formed H_2O_2 reacts with 4-aminophenazone and N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the presence of peroxidase (POD) to produce a chromophore, which is read at 660/800 nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample.

$$\text{Triglycerides} + 3\ H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + 3\ \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK, } Mg^{2+}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} H_2O_2 + \text{Dihydroxyacetone phosphate}$$

$$2\ H_2O_2 + \text{MADB} + \text{4AAP} \xrightarrow{\text{Peroxidase}} \text{Blue Dye} + OH^- + H_2O$$

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Serum triglyceride is stable for seven days when stored at 2 - 8°C and 3 months when stored frozen at ≤ -20°C.[7]

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Fasting (≥ 12 hours) serum samples,[8] free from hemolysis and removed from the clot are the recommended specimens. K2/K3 EDTA and Li/Na heparin are the suggested anticoagulants if plasma must be used.

Ensure that all equipment used in the collection and storage of samples is free from glycerol contamination.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Triglyceride Reagent

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. Dispose of all waste material in accordance with local guidelines.
3. This product contains material of animal origin. The product should be considered as potentially capable of transmitting infectious diseases.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

PIPES buffer (pH 7.5)	50 mmol/L
Lipase (Pseudomonas)	≥ 1.5 kU/L (25 µkat/L)
Glycerol kinase (Bacillus stearothermophilus)	≥ 0.5 kU/L (8.3 µkat/L)
Glycerol phosphate oxidase (Pseudomonas)	≥ 1.5 kU/L (25 µkat/L)
Ascorbate oxidase (Curcubita species)	≥ 1.5 kU/L (25 µkat/L)
Peroxidase (Horseradish)	≥ 0.98 kU/L (16.3 µkat/L)
ATP	1.4 mmol/L
4-Aminoantipyrine	0.50 mmol/L
Magnesium acetate	4.6 mmol/L
MADB	0.25 mmol/L
Also contains preservatives.	

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Not classified as hazardous

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

Chemistry Calibrator (Cat # DR0070)

Storage location of the Calibrator in this laboratory:

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

OSR66118 for use on the AU5800 systems only.

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The Triglyceride Reagents are ready for use. No preparation is required.

STORAGE AND STABILITY

1. The unopened reagents are stable until the expiration date printed on the label when stored at 2 – 8°C.
2. Opened reagents are stable for 30 days when stored in the refrigerated compartment of the analyzer.
3. A very fine suspension of particles which may settle out on storage may be evident in the R1 component of this reagent. The reagent can be used without effect to results.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, gross turbidity, precipitate or change in color in the Triglyceride reagent may indicate degradation and warrant discontinuance of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATION INFORMATION

The frequency of calibration is every 30 days. Calibration of the Triglyceride procedure is accomplished by use of Chemistry Calibrator (Cat # DR0070). For Traceability information refer to the calibrator instructions for use.

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.
3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (mmol/L) the result is multiplied by 0.0113.

REPORTING RESULTS

EXPECTED RESULTS

Triglyceride	Risk Classification[9]
<150 mg/dL	Normal
150-199 mg/dL	Borderline High
200-499 mg/dL	High
≥500 mg/dL	Very High

Reference Intervals shown above were taken from the literature. Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS (mg/dL)

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Results of studies[10] show that the following substances interfere with this triglyceride procedure.

The criteria for no significant interference is recovery within 10% of the initial value.

Ascorbate:	No significant interference up to 20 mg/dL Ascorbate
Bilirubin:	No significant interference up to 40 mg/dL Bilirubin
Hemolysis:	No significant interference up to 500 mg/dL Hemolysate

Venipuncture immediately after or during the administration of Metamizole (Dipyrone) may lead to falsely low results for Triglyceride. Venipuncture should be performed prior to the administration of Metamizole.

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[11]

Laboratory specific procedure notes:

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

This Triglyceride procedure is linear from 10 to 1,000 mg/dL. Samples exceeding the upper limit of linearity should be diluted and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

Note: Triglycerides GPO enzymatic methodologies are subject to a strong negative interference from patient samples with extremely elevated triglyceride levels.[12] While these samples are extremely lipemic in appearance and typically have triglyceride levels exceeding 1,700 mg/dL, results can be erroneously reported as being within the linear range of the assay. In order to identify grossly lipemic samples exhibiting this phenomenon, Data Check Parameters are provided. If the reaction kinetics of a test exhibits the characteristics of one of these elevated triglyceride samples, the analysis result will be flagged (F, Z, @ or &). Grossly lipemic samples under rare circumstances may evade the Data Check Parameters and should routinely be diluted 1 part sample to 4 parts saline prior to analysis and the results multiplied by 5.

ANALYTICAL SENSITIVITY

The lowest detectable level using serum settings on an AU analyzer was calculated as 0.81 mg/dL.

The lowest detectable level represents the lowest measurable level of triglyceride that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte free sample.

Limit of Quantitation

The Limit of Quantitation (LOQ) using serum settings for the Triglyceride reagent was determined to be 5 mg/dL. This was determined according to CLSI protocol EP17-A2[13] and represents the lowest concentration of triglyceride that can be measured with a total imprecision of 20%.

METHODS COMPARISON

Reference[14]

Patient serum samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	0.987
Intercept	2.563

Correlation Coeff. (r)	0.9999
No. of Samples (n)	115
Range (mg/dL)	27.20 - 937.84

PRECISION

Reference[14]

Estimates of precision, based on CLSI recommendations,[15] are consistent with typical performance. The within run precision is less than 3% CV and total precision is less than 5% CV. Assays of serum pools were performed and the data reduced following CLSI guidelines above.

N = 80	Within-run		Total	
Mean, mg/dL	**SD**	**CV%**	**SD**	**CV%**
51	0.30	0.6	0.50	1.0
206	0.85	0.4	0.98	0.5
815	2.68	0.3	3.81	0.5

ADDITIONAL INFORMATION

DxC 700 AU analyzers require that each reagent application has a standard format of abbreviated Test Name. This Test Name is required to allow automated loading of the calibrator information for each application. Refer to the table below for the Test Name assigned to each application for this assay.

Test Name	Description
TRG1U	Triglyceride (Serum)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle

† Beckman Coulter System Calibrator Cat No.: DR0070

* Values set for working in mg/dL. To work in SI units (mmol/L) multiply by 0.0113

REVISION HISTORY

Updated Specimen Section

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

Preceding version revision history

Add DxC 500i instrument to IFU

REFERENCES

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