Infinity™
Triglycerides Liquid Stable Reagent

PRODUCT SUMMARY

Stability	:	18 Months at 2-8°C
Linear Range	:	Up to 10 mmol/L (885 mg/dL)
Specimen Type	:	Serum or plasma
Method	:	Endpoint
Reagent Preparation	:	Supplied ready to use.

IVD Rx ONLY

SYMBOLS IN PRODUCT LABELLING

EC REP	Authorised Representative		Temperature Limitation
IVD	For in vitro diagnostic use		Use by/Expiration Date
LOT	Batch code/Lot number		CAUTION. CONSULT INSTRUCTIONS FOR USE.
REF	Catalogue number		Manufactured by
	Consult instructions for use		
Rx ONLY	Prescription Use Only		

INTENDED USE
This reagent is intended for the in vitro quantitative determination of Triglycerides in human serum or plasma.

CLINICAL SIGNIFICANCE
Triglycerides are a family of lipids absorbed from the diet and produced endogenously from carbohydrates. Measurement of triglycerides is important in the diagnosis and management of hyperlipidaemias. These diseases can be genetic or secondary to other disorders including nephrosis, diabetes mellitus, and endocrine disturbances. Elevation of triglycerides has been identified as a risk factor for atherosclerotic disease[1].

METHODOLOGY
This reagent is based on the method of Wako[2] and the modifications by McGowan et al[3] and Fossati et al.[4]

1. Triglycerides + H_2O $\xrightarrow{Lipase}$ Glycerol + Free Fatty acids
2. Glycerol + ATP $\xrightarrow{GK}$ Glycerol-3-phosphate + ADP
3. Glycerol-3-phosphate + O_2 $\xrightarrow{GPO}$ DAP + $2H_2O_2$
4. H_2O_2 + 4-AAP + 3,5 DHBS $\xrightarrow{POD}$ Quinoneimine dye + $2H_2O$

1. Triglycerides are enzymatically hydrolysed by lipase to free fatty acids and glycerol.
2. The glycerol is phosphorylated by adenosine triphosphate (ATP) with glycerol kinase (GK) to produce glycerol-3-phosphate and adenosine diphosphate.
3. Glycerol-3-phosphate is oxidised by dihydroxyacetone phosphate (DAP) by glycerolphosphate oxidase producing hydrogen peroxide (H_2O_2).
4. In a Trinder[5] type colour reaction catalyzed by peroxidase, the H_2O_2 reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DHBS) to produce a red coloured dye. The absorbance of this dye is proportional to the concentration of triglycerides present in the sample.

REAGENT COMPOSITION

Active Ingredients	Concentration
ATP	2.5 mmol/L
Mg Acetate	2.5 mmol/L
4 - Aminoantipyrine	0.8 mmol/L
DHBS	1.0 mmol/L
GPO (microbial)	> 3000 U/L
Glycerol Kinase (microbial)	> 100 U/L
Lipoprotein Lipase (microbial)	> 2000 U/L
Peroxidase (horseradish)	> 300 U/L
Buffer	53 mmol/L
pH 7.0 ± 0.1 at 20°C	

WARNING: Do not ingest. Avoid contact with skin and eyes. If spilt, thoroughly wash affected areas with water. Reagent contains sodium azide which may react with copper or lead plumbing. Flush with plenty of water when disposing. For further information consult the Infinity Triglycerides Liquid Stable Reagent Material Safety Data Sheet.

CAUTION: This product contains animal source material. Handle and dispose of this product as if it were potentially infectious.

REAGENT PREPARATION
The reagent is supplied ready to use.

STABILITY AND STORAGE
When stored at 2 - 8°C, the reagent is stable until the expiration date stated on the bottle and kit box label.

Indications of Reagent Deterioration:
- Turbidity;
- Reagent Absorbance >0.2 AU (520 nm, 1cm lightpath); and/or
- Failure to recover control values within the assigned ranges.

SPECIMEN COLLECTION AND HANDLING
Collection: Blood for Triglycerides estimation should be collected after a 10-14 hour fast.[1] As variation in Triglycerides estimation is due to both analytical and biological variation, before treatment decisions are finalised, it is recommended that 3 samples taken at least 1 week apart, are assayed.[6]
Serum: Use non-haemolysed serum. Blood collection tubes with glycerol lubricated stoppers should not be used.[1]
Plasma: Heparinised plasma is a suitable specimen.
Storage: Triglycerides are stable for 3 days at 4°C and several weeks at -20°C. For longer periods specimens should be stored at -70°C. Storage at room temperature may cause the release of glycerol from phospholipids with a resulting apparent increase in Triglycerides and hence is not recommended. Lipaemic specimens, if they have been frozen may require warming to 37°C and vigorous mixing prior to use.[1]

ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED
- A clinical chemistry analyser capable of maintaining constant temperature (37°C) and measuring absorbance between 500 and 550nm.
- Analyser specific consumables, eg: samples cups.
- If required, pipettes for accurately dispensing measured volumes.
- Normal and Abnormal assayed controls.
- Calibrator or a suitable aqueous Triglycerides standard.

ASSAY PROCEDURE
The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

SYSTEM PARAMETERS

Temperature	37°C
Primary Wavelength	500 nm (500-550nm)
Secondary Wavelength	660 nm (600-660nm)
Assay Type	Endpoint
Direction	Increase
Sample : Reagent Ratio	1:100
eg: Sample Vol	3 µL
Reagent Vol	300 µL
Incubation Time	300 seconds
Reagent Blank Limits	Low 0.0 AU
(500nm, 1cm lightpath)	High 0.2 AU
Linearity	10 mmol/L (885 mg/dL)
Analytical Sensitivity	0.158 ΔA per mmol/L
(500 nm, 1cm lightpath)	(0.002 ΔA per mg/dL)

CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

$$\text{Triglycerides} = \frac{\text{Absorbance of Unknown}}{\text{Absorbance of Calibrator}} \times \text{Calibrator Value}$$

Example:
Absorbance of Calibrator = 0.164
Absorbance of unknown = 0.113
Value of Calibrator = 2.9 mmol/L (257 mg/dL)

$$\text{Triglycerides} = \frac{0.113}{0.164} \times 2.9 = 2.0 \text{ mmol/L}$$

$$\text{Triglycerides} = \frac{0.113}{0.164} \times 257 = 177 \text{ mg/dL}$$

NOTES
1. Specimens assayed with triglycerides values greater than 10 mmol/L (885 mg/dL) should be diluted with saline and reassayed. Multiply the result by the dilution factor.
2. The colour reaction is stable for at least 10 minutes at 37°C.
3. Unit conversion: mmol/L x 88.5 = mg/dL

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CALIBRATION[1,7]

Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. Aqueous glycerol standards can be used, however, glycerol can only be considered a primary standard for the indicator system, as it does not participate in the first reaction step. A serum based secondary calibrator, with a value close to 2.25 mmol/L (200 mg/dL) is recommended.

For calibration frequency on automated instruments, refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert.

Recalibration is recommended at anytime if one of the following events occurs:

- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL

To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-

- At least once per day or as established by the laboratory.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate that the assay may be out of control. The following corrective actions are recommended in such situations:-

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are sill out of control, perform a calibration with freshly prepared reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS

1. Glycerol contamination will affect this assay, which may result in the misclassification of a patients risk status. As a result, the American Associations of Clinical Chemistry has made a series of recommendations regarding glycerol blanking which can be found in Reference 1.
2. Studies to determine the level of interference from bilirubin (free & conjugated), haemoglobin and ascorbic acid were carried out using commercially available interference check products. The following results were obtained:
   Haemoglobin: No interference from haemoglobin up to a level of 1000 mg/dL.
   Free Bilirubin: No interference from free bilirubin up to a level of 58 µmol/L (3.4 mg/dL).
   Conjugated Bilirubin: No interference from conjugated bilirubin up to a level of 51 µmol/L (3 mg/dL).
   Ascorbic Acid: No interference from ascorbic acid up to a level of 2.0 mg/dL (0.114 mmol/L).
3. Young DS[8] has published a comprehensive list of drugs and substances which may interfere with this assay.

EXPECTED VALUES

Recommended (desirable) Triglycerides levels for adults:[1]

Male:	0.45 - 1.81 mmol/L	40 - 160 mg/dL
Female:	0.40 - 1.53 mmol/L	35 - 135 mg/dL

The NIH consensus conference[6] classified hypertriglycerideaemia into two categories.

Distinct hypertriglycerideaemia: Triglyceride >5.6 mmol/L (>500 mg/dL)
Borderline hypertriglycerideaemia: Triglyceride value 2.8 - 5.6 mmol/L (250 - 500 mg/dL).

PERFORMANCE DATA

The following data was obtained using the Infinity Triglycerides Liquid Stable Reagent on a well maintained automated clinical chemistry analyser. Users should establish product performance on their specific analyser used.

IMPRECISION

Within Run:	LEVEL I	LEVEL II
Number of samples	15	15
Mean (mmol/L / mg/dL)	1.11 / 98.3	1.86 / 164.7
SD (mmol/L / mg/dL)	0.02 / 1.77	0.02 / 1.77
CV (%)	2.07	1.25

Total:	LEVEL I	LEVEL II
Number of samples	40	40
Mean (mmol/L / mg/dL)	1.12 / 98.8	1.72 / 152.1
SD (mmol/L / mg/dL)	0.05 / 4.4	0.03 / 2.5
CV (%)	4.5	1.6

METHOD COMPARISON

Comparison studies were carried out on an automated clinical chemistry analyser using a similar commercially available Triglycerides reagent as a reference. Serum samples were assayed in parallel and the results compared by the least regression. The following statistics were obtained.

Number of sample pairs	40
Range of sample results	1.06 - 4.06 mmol/L (93.8 - 359.3 mg/dL)
Mean of reference method results	1.93 mmol/L (170.8 mg/dL)
Mean of Triglycerides results	2.01 mmol/L (177.9 mg/dL)
Slope	0.96
Intercept	0.22 mmol/L (19.5 mg/dL)
Correlation Coefficient	0.995

LINEARITY

When run as recommended the assay is linear up to 10 mmol/L (885 mg/dL).

ANALYTICAL SENSITIVITY

When run as recommended the sensitivity of this assay is 0.158 ΔA per mmol/L or 0.002 ΔA per mg/dL (1cm lighpath, 500 nm).

REFERENCES

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REF

Reorder Information

Catalogue No.	Configuration
TR22421	2 x 125 mL