

### Diagnostic reagent for quantitative in vitro determination of triglycerides in serum or plasma on photometric systems

#### Order Information

| Cat. No.            | Kit size                         |
|---------------------|----------------------------------|
| 01 00098 70 04 0125 | R: 5 x 25 mL                     |
| 01 00098 70 04 0600 | R: 6 x 100 mL                    |
| 01 00098 70 02 0240 | R: 4 x 60 mL                     |
| 01 00098 70 10 0160 | R: 4 x 40 mL                     |
| CDT-TG              | R: 4 x 30mL                      |
| 06 00116 70 04 0018 | Triglycerides Standard<br>6x3 mL |

#### Summary [1,2]

Triglycerides are esters of glycerol with three fatty acids and are the most abundant naturally occurring lipids. They are transported in plasma bound to apolipoproteins forming very low density lipoproteins (VLDL) and chylomicrons. Measurement of triglycerides is used in screening of the lipid status to detect atherosclerotic risks and in monitoring of lipid lowering measures. Studies have shown that elevated triglyceride concentrations combined with increased low density lipoprotein (LDL) concentrations constitute an especially high risk for coronary heart disease (CHD). High triglyceride levels also occur in various diseases of liver, kidneys and pancreas

#### Method

Colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO)

#### Principle

Determination of triglycerides after enzymatic splitting with lipoprotein lipase. Indicator is quinoneimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase.

Triglycerides  $\xrightarrow{\text{LPL}}$  Glycerol + fatty acid

Glycerol + ATP  $\xrightarrow{\text{GK}}$  Glycerol-3-phosphate + ADP

Glycerol-3-phosphate + O<sub>2</sub>  $\xrightarrow{\text{GPO}}$  Dihydroxyacetone phosphate + H<sub>2</sub>O<sub>2</sub>

2 H<sub>2</sub>O<sub>2</sub> + Aminoantipyrine + 4-Chlorophenol  $\xrightarrow{\text{POD}}$  Quinoneimine + HCl + 4 H<sub>2</sub>O

#### Reagent

##### Components and Concentrations

|                              |        |                        |
|------------------------------|--------|------------------------|
| Good's buffer                | pH 7.2 | 50 mmol/L              |
| 4-Chlorophenol               |        | 4 mmol/L               |
| ATP                          |        | 2 mmol/L               |
| Mg <sup>2+</sup>             |        | 15 mmol/L              |
| Glycerokinase                | (GK)   | ≥ 0.4 kU/L             |
| Peroxidase                   | (POD)  | ≥ 2 kU/L               |
| Lipoprotein lipase           | (LPL)  | ≥ 2 kU/L               |
| 4-Aminoantipyrine            |        | 0.5 mmol/L             |
| Glycerol-3-phosphate-oxidase | (GPO)  | ≥ 0.5 kU/L             |
| <b>Standard:</b>             |        | 200 mg/dL (2.3 mmol/L) |

#### Storage Instructions and Reagent Stability

Reagent and standard are stable up to the end of the indicated month of expiry, if stored at 2 – 8°C, protected from light and contamination is avoided. Do not freeze the reagent!

**Note:** It has to be mentioned, that the measurement is not influenced by occasionally occurring color changes, as long as the absorbance of the reagent is < 0.3 at 546 nm.

#### Warnings and Precautions

1. The reagent contains sodium azide (0.95 g/L) as preservative. Do not swallow! Avoid contact with skin and mucous membranes.
2. The reagent contains biological material. Handle the product as potentially infectious according to universal precautions and good laboratory practice.
3. In very rare cases, samples of patients with gammopathy might give falsified results [6].
4. N-acetylcysteine (NAC), acetaminophen and metamizole medication leads to falsely low results in patient samples.
5. Please refer to the safety data sheets and take the necessary precautions for the use of laboratory reagents. For diagnostic purposes, the results should always be assessed with the patient's medical history, clinical examinations and other findings.
6. For professional use only!

#### Waste Management

Please refer to local legal requirements.

#### Reagent Preparation

The reagent and the standard are ready to use.

#### Materials required but not provided

NaCl solution 9 g/L  
General laboratory equipment

#### Specimen

Serum, heparin plasma or EDTA plasma

|                |                   |    |            |
|----------------|-------------------|----|------------|
| Stability [4]: | 2 days            | at | 20 - 25 °C |
|                | 7 days            | at | 4 - 8 °C   |
|                | at least one year | at | - 20 °C    |

Discard contaminated specimens. Freeze only once!

#### Assay Procedure

*Application sheets for automated systems are available on request.*

|              |                       |
|--------------|-----------------------|
| Wavelength   | 500 nm, Hg 546 nm     |
| Optical path | 1 cm                  |
| Temperature  | 20-25 °C / 37 °C      |
| Measurement  | Against reagent blank |

|  | Blank   | Sample or standard |
|--|---------|--------------------|
| <b>Sample or standard</b>                              | -       | 10 µL              |
| <b>Dist. water</b>                                     | 10 µL   | -                  |
| <b>Reagent</b>   | 1000 µL | 1000 µL            |
| Mix, incubate 20 min at 20 - 25 °C or 10 min at 37 °C. |         |                    |
| Read absorbance against the blank within 60 min.       |         |                    |

### Calculation

#### With standard or calibrator

$$\text{Triglycerides [mg/dL]} = \frac{A \text{ Sample}}{A \text{ Std/Cal}} \times \text{Conc. Std/Cal [mg/dL]}$$

To correct for free glycerol, subtract 10 mg/dL (0.11 mmol/L) from the triglycerides value calculated above.

#### Conversion factor

$$\text{Triglycerides [mg/dL]} \times 0.01126 = \text{Triglycerides [mmol/L]}$$

### Calibrators and Controls

For the calibration of automated photometric systems, the DiaSystem UniCal CC is recommended. The assigned values of UniCal CC have been made traceable to the reference method gas chromatography-isotope dilution mass spectrometry (GC-IDMS). DiaSystem UniLab N and P or DiaSystem UniLab Lipid controls should be assayed for internal quality control. Each laboratory should establish corrective action in case of deviations in control recovery.

|                      | Cat. No.            | Kit size |
|----------------------|---------------------|----------|
| UniCal CC            | 06 00122 70 04 0018 | 6 x 3 mL |
| UniLab N             | 07 00123 70 05 0030 | 6 x 5 mL |
| UniLab P             | 07 00124 70 05 0030 | 6 x 5 mL |
| UniLab Lipid Level 1 | 07 00129 70 04 0009 | 3 x 3 mL |
| UniLab Lipid Level 2 | 07 00130 70 04 0009 | 3 x 3 mL |

### Performance Characteristics

#### Measuring range

The test has been developed to determine triglyceride concentrations within a measuring range from 2 - 1000 mg/dL (0.02 - 11.3 mmol/L). When values exceed this range, samples should be diluted 1 + 4 with NaCl solution (9 g/L) and the result multiplied by 5.

#### Specificity/Interferences

No interferences were observed by ascorbic acid up to 3 mg/dL, conjugated bilirubin up to 30 mg/dL, by unconjugated bilirubin up to 9 mg/dL and hemoglobin up to 500 mg/dL. For further information on interfering substances refer to Young DS [5].

#### Sensitivity/Limit of Detection

The lower limit of detection is 2 mg/dL.

#### Precision (at 37°C)

| Intra-assay precision<br>n = 20 | Mean<br>[mg/dL] | SD<br>[mg/dL] | CV<br>[%] |
|---------------------------------|-----------------|---------------|-----------|
| Sample 1                        | 55.5            | 0.301         | 0.54      |
| Sample 2                        | 212             | 1.69          | 0.80      |
| Sample 3                        | 447             | 3.09          | 0.69      |

| Inter-assay precision<br>n = 20 | Mean<br>[mg/dL] | SD<br>[mg/dL] | CV<br>[%] |
|---------------------------------|-----------------|---------------|-----------|
| Sample 1                        | 88.9            | 0.795         | 0.89      |
| Sample 2                        | 235             | 3.61          | 1.54      |

#### Method Comparison

A comparison of DiaSystem Triglycerides (y) with a commercially available test (x) using 95 samples gave following results:  $y = 0.969x - 0.092$  mg/dL;  $r = 0.9999$

### Reference Range [2]

|                  |                                    |
|------------------|------------------------------------|
| Desirable:       | < 200 mg/dL (fasting) (2.3 mmol/L) |
| Borderline high: | 200 – 400 mg/dL (2.3 – 4.5 mmol/L) |
| Elevated:        | > 400 mg/dL (4.5 mmol/L)           |

Each laboratory should check if the reference ranges are transferable to its own patient population and determine own reference ranges if necessary.

### Clinical Interpretation [3]

Epidemiological studies have observed that a combination of plasma triglycerides > 180 mg/dL (> 2.0 mmol/L) and HDLcholesterol < 40 mg/dL (1.0 mmol/L) predict a high risk of CHD. Borderline levels (> 200 mg/dL) should always be regarded in association with other risk factors for CHD.

### Literature

1. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3<sup>rd</sup> ed. Philadelphia: W.B Saunders Company; 1999. p. 809-61.
2. Cole TG, Klotzsch SG, McNamara J. Measurement of triglyceride concentration. In: Rifai N, Warnick GR, Dominiczak MH, eds. Handbook of lipoprotein testing. Washington: AACC Press, 1997. p. 115-26.
3. Recommendation of the Second Joint Task Force of European and other Societies on Coronary Prevention. Prevention of coronary heart disease in clinical practice. Eur Heart J 1998; 19: 1434-503.
4. Guder WG, Zawta B et al. The Quality of Diagnostic Samples. 1<sup>st</sup> ed. Darmstadt: GIT Verlag; 2001; p. 46-7.
5. Young DS. Effects of Drugs on Clinical Laboratory Tests. 5th ed. Volume 1 and 2. Washington, DC: The American Association for Clinical Chemistry Press 2000.
6. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007; 45(9):1240-1243.

### Manufacturer

DiaSystem Scandinavia AB  
Datorgatan 3, Sweden – 561 33 Jönköping  
Phone +46 36 126220 ▪ Fax +46 36 187730  
info@diasystem.se ▪ www.diasystem.se

