TOTAL LIPID REAGENT SET
(COLORIMETRIC METHOD)

IVD *For In-Vitro diagnostic and professional use only*

2°C 8°C Store at 2-8 °C CE

INTENDED USE

For the quantitative determination of the total lipid index in serum or plasma using the sulfo-phospho-vanillin colorimetric method.

INTRODUCTION

The lipids are a large and diverse group of naturally occurring organic compounds that are related by their solubility in nonpolar organic solvents (e.g. ether, chloroform, acetone & benzene) and general insolubility in water. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology.

Lipids react with sulfuric acid to form carbonium ions, which subsequently react with the vanillin phosphate ester to yield a purple complex that is measured photometrically.

In blood, at least 95% of the lipids exist in combination with protein. These lipoproteins can be quantitated by disrupting this complex. This test is used as a screening method for hyperlipidemia. The sulfo-phospho-vanillin (SPV) method for the colorimetric determination of the serum total lipids was described by Charbrol et al[2] and modified by several investigators by omitting phosphoric acid, shortening reaction time, and increasing reagent stability.Our reagent is based on all of these modifications.

PRINCIPLE

Unsaturated lipids react with sulphuric acid to form carbonium ions. In a second step the carbonium ions react with phosphovainilline to give a pink colour. The intensity of the color formed is proportional to the total lipids concentration in the sample.

MATERIALS

REAGENTS

Reagent	Phosphovainilline Phosphoric acid	235 mmol/L 2 mmol/L
Standard	Total Lipids aqueous primary standard 750 mg/dL	
Sulfuric Acid	Needed reagent but not provided.	

MATERIALS REQUIRED BUT NOT PROVIDED

- Concentrated sulfuric acid.
- Pipettes.
- Test vials or cuvettes.
- Timer.
- 100°C heating bath.
- Control serum.
- Spectrophotometer.
- Sulphuric Acid (H_2SO_4).

PREPARATION

Reagent and standard are ready to use.

PRECAUTIONS

- Exercise the normal precautions required for handling of all laboratory reagents.
- Pipetting by mouth is not recommended for any laboratory reagent. Concentrated sulfuric acid causes severe burns and eye damage.

STORAGE AND STABILITY

- All reagents are stable until the expiration date indicated on label.
- Total lipid Reagent Store at 2-8°C.
- Protected from light.
- Stability of the sample: Total lipids are stable 24 h at room temperature (15-25ºC) or 3 days at 2-8ºC.
- Signs of reagent deterioration:
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 520 nm ≥0.32.

SPECIMEN COLLECTION

- Serum or Plasma.

PROCEDURES

1. Assay conditions:
   Wavelength ..... 520 nm (490-550).
   Cuvette: .....1 cm light path.
   Temperature .....37°C.
2. Adjust the instrument to zero with distilled water.
3. Pipette into a glass tube:

Table 1	Standard	Sample
H_2SO_4 (mL)	2.5	2.5
Standard (µL)	100	-
Sample (µL)	-	100

4. Mix and incubate for **10 minutes** at 100°C in a boiling water bath.

➢ **Note:**

- H_2SO_4 with Standard give Standard Acid digest **(Solution A)**.
- H_2SO_4 with Sample give Sample Acid digest (**Solution B**).

5. Cool in iced water and transfer from (Table 1) into a cuvette in (Table 2):

Table 2	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Sample Acid digest (µL) (**Solution B**)	-	-	50
Standard Acid digest (µL) (**Solution A**)	-	50	-

6. Mix and incubate for exactly 1**5 minutes** at 37°C.
7. Read the absorbance (A) of the samples and standard, against the Blank. The color is stable for at least 1 hour.

CALCULATIONS:

(A) Sample - (A) Blank) **X** 750 (Conc. of STD) =
(A) Standard - (A) Blank

Total Lipid in sample (mg/dl)

REFERENCE VALUES

Serum or Plasma:
450-800 mg /dL
These values are for orientation purpose; each laboratory should establish its own reference range.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.
If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 7.7 mg/dL to linearity limit of 1500 mg/dL.
If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.

Precision:

	Intra-assay (n=20)	
Mean (U/L)	555	919
SD	15.9	6.47
CV (%)	2.87	0.70
	Inter-assay (n=20)	
Mean (U/L)	553	919
SD	7.62	5.87
CV (%)	1.78	0.63

Sensitivity:
1 mg/dL = 0, 00066 A.

Accuracy:
Results obtained using ATLAS reagents (y) did not show systematic differences when compared with other commercial reagents (x). The results obtained using 50 samples were the following: Correlation coefficient (r): 0,984 .
Regression equation: y=0,967x + 24, 08.
The results of the performance characteristics depend on the analyzer used.

NOTES

- TOTAL LIPIDS CAL: Proceed carefully with this product because due its nature it can get contamined easily.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.

REFERENCES

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4. Frings, CS and Dunn, RT, Am J Clin. Path., 53:89 (1970).
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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry