Total Urinary Protein – Method SDS
Instructions for use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB NDIKO	REF B75282582	n° 16 vials x 20 mL

INTENDED USE
For the *in vitro* quantitative determination of the total protein concentration in human urine. The results of the test must always be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE
Thousands of proteins are contained in body fluids for different purposes.
Many proteins are structural elements of cells or organized tissues. Other proteins are soluble and they are free molecules moving in intracellular or extracellular fluids.
The glomerular basement membrane acts as an ultra filter for plasma proteins, which pass through the membrane in function of molecular size, net ionic charge, plasma concentration. All small proteins, passing through the glomerular ultra filter, are reabsorbed by the renal tubules.
In general, transport of protein molecules through the glomerular membrane is inversely related to size, net negative charge and hydrodynamic ray. A given amount of proteins excreted with urines also depends on the extent of its reabsorption by the renal tubules and inversely related to their molecular size.
Proteinuria may be defined as an increase in the amount of proteins in urine. Types include glomerular, tubular, overload, and post renal proteinuria, each characterized by a given amount of proteins.
Glomerular proteinuria is the most common and serious type of abnormal proteinuria. Because most of the excreted proteins is albumin, glomerular proteinuria is often labelled albuminuria. Tubular proteinuria, due to tubules impairment, is not less important than the glomerular one, only being less widely known.
Even small increasing levels of Total Proteinuria over the upper limit of reference range, require further investigation, in order to exclude any glomerular or tubular diseases. Overload proteinuria might be due to Mieloma. If you suspect such a disease, a urine electrophoresis must be performed.

PRINCIPLE OF THE METHOD
Pyrogallol – Molybdate SDS Method
The combination of Red-Molybdate Pyrogallol creates a bond with the proteins present in the sample. This link creates an absorption peak at 600 nm and the formation of colour is directly proportional to the concentration of proteins.

Preservation and stability
= storage temperature 15-25°C
If stored closed at the indicated temperature, avoiding direct light, the intact reagents are stable until the expiration date, printed on the label.

Concentrations

Reagent:			
	Conc.	U.M.	
Succinic Acid	50	mM	
Sodium Oxalate	1,04	mM	
Sodium Benzoate	3,5	mM	
Sodium Molibdate	40	µM	
Tensioactive	0,5	g/L	GHS08 *
Sodium dodecyl sulfate	0,1	mM	
Methanol	40	mM	
Pyrogal Red	0,06	mM	

* _ Wearning: **DAINGER**
H371 - May cause damage to organs.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P264 - Wash thoroughly after handling.
P270 - Do not eat, drink or smoke when using this product.
P308+P311 - IF exposed or concerned: Call a POISON CENTER/doctor.
P405 - Store locked up.
P501 - Dispose of contents/container in accordance with local/regional/national/international regulations.

Reagents included in the kit
The reagent is described above.

Materials required but not supplied in the kit
Controls, calibrators and pipettes with adequate volume.

PRECAUTIONS and WARNINGS
1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents
In the event of a serious incident in relation to the use of the device, please inform the manufacturer (via your distributor) and the competent authority of the European Union member state where the incident occurred. For other jurisdictions, reporting must be made in accordance with regulatory requirements.
Reporting serious incidents helps provide more information about the safety of the diagnostic medical device.

PROCEDURE
Quality Control
Known controls containing PTUs are commercially available for quality control, accompanied by certificates of analysis. High/Low Sclavo Diagnostics is available, Code B35282592. The values obtained must be contained within the acceptability range or your CQI.

Calibration
For calibration use the Sclavo "PTU Calibrator" kit Code B35282591.

Traceability
The PTU value is visible in the insert of the calibration serum package.

SAMPLE
Type of sample and storage
Urine samples should be used. Store out of the light. Urine samples can be stored for 1 day at 20 - 25 ° C, 7 days at 2-8 ° C or 1 month at -20° C. However, the sample should be processed as soon as possible, as it is easily contaminable.

PREPARATION OF THE REAGENT
Liquid reagent ready to use. After opening the reagent is stable 30 days when closed and protected from direct light. Slight variations in composition among batches will not affect test results.

Automation
The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD
The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction Conditions
Wavelength (primary): 600 nm
Temperature: 37°C

Technique
- Bring the reagents to room temperature and operate away from direct light.
- Reaction volumes can be varied proportionally.

	U.M.	Blank.	Calib. Serum	Sample
Reagent	µL	200	200	200
Calib. Serum	µL	-	3,00	-
Sample	µL	-	-	3,00
Distilled Water	µL	3,00	-	-

Mix well and after 3 minutes carry out reading at 37°C.

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Read the absorbance of sample (O.D. sample) and calibrator (O.D. calibr.) against reagent blank (complete reading within 10 minutes).
The reaction volumes may be varied proportionally without alteration of results.

Results:
Manual Method
Calculation of PTU concentration:

$$\frac{\text{Sample O.D.}}{\text{Calibrator O.D.}} \times \text{Calibrator Concentration} = \text{PTU mg/dL}$$

Automation
The results are automatically calculated by the analyzer based on the calibration curve/line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration curve/line is obtained through a special validated algorithm.

Reference Interval
URINE
- 28 – 141 mg/24h

Every laboratory must set its own normal-range values based on the population under study.

CHARACTERISTICS/PERFORMANCE
Linearity
The reaction is linear up to 300 mg / dL. If the value in the sample exceeds the linearity limit of the method, dilute the sample with saline and multiply the result for the dilution factor.

Specificity
The specificity of the method PTU is documented by the close correlation with electrophoretic patterns as evidenced in the literature.

Accuracy - Recovery
Accuracy studies have been carried out on normal samples to which albumin of known titre was added. The data indicate a recovery of 99% up to concentrations of 3 mg / dL.

Interference
Interferences has been observed due to the presence of strong acids or bases. Common substances present in urine may interfere to a maximum of 2%.

Precision of the method

Accuracy in the series (Within-run precision) – Repeatability					
Range	U.M.	Mean	,S.D.	C.V. (%)	N°
Low	mg/dL	11,84	0,189	1,6	30
High	mg/dL	71,24	1,023	1,4	30
Total precision (Within-lab precision)					
Range	U.M.	Mean	S.D.	C.V. (%)	N°
Low	mg/dL	11,60	0,54	4,70	20
High	mg/dL	70,88	1,40	1,98	20

Limits of sensitivity
The Sensitivity limit has been measured using serial dilutions of high concentrated sera. The smallest detectable concentration is about 2.27 mg / dL of PTU in the conditions established for this test.

Comparison between methods
The Sclavo method of total urinary proteins was compared with a similar commercially available method, analyzing 72 human urines. The correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	3.63
Slope	1.1318
Correlation Coeff. (R)	0.9981

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

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REVISION	DATE	CHANGE
Rev.B	05/2025	New Issue for IVDR Regulation (UE) 2017/746 compliance

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