Instructions for Use
suPARnostic® TurbiLatex Reagents
Soluble Urokinase Plasminogen Activator Receptor

REF **T001**

CE IVD

ViroGates A/S
Blokken 45
Birkerod 3460, Denmark
Tel: +45 2113 1336
Skype: Virogates
www.virogates.com

This est is validated on Roche Cobas **c111**
(Cobas® is a trademark of Roche)

Refer to the webpage http://www.virogates.com for application notes for other biochemistry analyzers and for instructions in other languages. Alternatively contact your local distributor for instructions in your language.

INTENDED USE
FOR PROFESSIONAL USE
The suPARnostic® TurbiLatex Reagents are used for determination of soluble urokinase plasminogen activator receptor (suPAR) in human EDTA- and heparin plasma and serum in ng/ml.

Interpretation of results must be made considering the patient´s clinical history and results of other diagnostic tests if available.

SUMMARY OF suPAR AS A MARKER OF DISEASE PROGNOSIS
suPAR is the soluble form of urokinase plasminogen activator receptor (uPAR). The amount of suPAR is a measure of immune activation and inflammation. suPAR is a non-specific biomarker which is increased by presence of disease. The higher the suPAR level, the higher the risk of disease progression and the worse the patient's prognosis.

PRINCIPLES OF ASSAY PROCEDURE
The suPARnostic® TurbiLatex test is a turbidimetric immunoassay that quantitatively determines suPAR in human plasma/serum samples. First stage of testing is an incubation of human origin specimen (EDTA, Heparin, Serum) with R1 reagent. After 5 min of incubation, R2 reagent is added and the reaction starts. Reaction buffer R2 is a suspension of latex particles coated with rat and mouse monoclonal antibodies to suPAR. After R2 addition the process of suPAR aggregation begins, the level of accumulation is determined by the amount of scattered light during measurement of light absorption. Linear calibration curve, created before the start of test, is used to determine the concentration of suPAR in human plasma samples.

REAGENTS AND MATERIALS
Reagents Provided
This kit contains ready-to-use 20 mL of R1 Dilution Buffer and 8 mL of R2 Solution of Latex particles coated with anti-suPAR antibodies. (These volumes are sufficient for 100 tests, one calibration and dead volume).

1. Reagent 1 – Dilution Buffer
2. Reagent 2 – Latex Particle Reagent
3. Instruction for use.

Material required but not provided

- suPARnostic® TurbiLatex Calibrators REF T002
- suPARnostic® TurbiLatex Controls REF T003
- Clinical chemistry analyser
- Adjustable pipette with tips, 20 µL – 200 µL
- Disposable gloves

REQUIRED TRAINING
To use the suPARnostic® TurbiLatex Reagents it is required that the user is fully trained in how to operate the chemistry analyzer.

REAGENT PRECAUTIONS AND RECOMMENDATIONS
- For professional users.
- Do not use kit components beyond the indicated kit expiration date.
- Do not switch caps on reagent containers as it may cause contamination or mix-up.
- Do not mix reagents from different kit lots.
- Do not freeze any of the kit components.
- Do not mouth pipette or ingest any of the reagents.
- Do not smoke, eat, or drink when performing the assay or in areas where samples or reagents are handled.
- Do not mix plasma samples from different patients or from different blood samplings of the same patient.
- Human samples may be contaminated with infectious agents. Do not ingest, expose to open wounds, or breathe aerosols. Wear protective gloves and dispose of biological samples properly.
- Be aware of possible dilution of suPAR in the case of transfusion, infusion or similar.
- All solutions supplied should be handled carefully and disposed of in accordance with national and local regulations.

STORAGE AND HANDLING
Store at 2-8°C. Before use check the expiry date on the label.
4 weeks of on-board stability when kept at 2-8°C in original containers.

SPECIMEN COLLECTION AND HANDLING

Sample Type	*Sample Requirement*
Plasma or serum sample	10 µl

SAMPLE COLLECTION AND STORAGE
Collecting blood samples should be performed by trained and qualified staff in antiseptic conditions using approved venepunctures techniques. Appropriate tube type should be chosen in accordance with the sample type used.
To prepare plasma samples, whole blood is drawn into a blood collection tube containing EDTA or Heparin anti-coagulant. Centrifuge the blood at 3,000 x g between 1 - 10 minutes.
Serum samples should be collected to proper tubes according

to standard procedure.

Do not use hemolyzed, contaminated or hyper-lipemic sample specimens.

SAMPLE STABILITY
It is preferred to analyze the samples as soon as possible but EDTA-, Heparin- and Serum samples are stable for:
24 hours at room temperature (20-25°C)
3 days at 2-8°C.
-20°C for longer storage. Five freeze/thaw cycles over 5 days do not have an impact on suPAR concentration in a sample.

NUMBER OF DETERMINATIONS
1 mL of R2 reagent is sufficient for 20 tests when measured on Roche Cobas® c111 analyser. This does not include dead volume in the bottles.

CALIBRATION
Together with suPARnostic® TurbiLatex Reagents the suPARnostic® TurbiLatex Calibrators T002 should be used for calibration. The laboratory shall work out their own frequency of calibration, but it is recommended to repeat the calibration at least once a month and it is required to recalibrate when a new batch of TurbiLatex Reagents are used.

QUALITY CONTROL
Quality control of TurbiLatex Reagents should be performed with suPARnostic® TurbiLatex Controls T003 after each calibration. Quality control ranges and limits should be fitted to the specific laboratory's experience and knowledge. If the values exceed established upper/lower range the laboratory should undertake corrective actions.

MEASURING RANGE
The measuring range of the suPARnostic® TurbiLatex Test is 1.5 ng/mL to 15.0 ng/mL on a Cobas® c111 analyser. Samples outside the quantitative range will be reported as suPAR < 1,5 ng/ml or suPAR > 15 ng/ml, respectively.

RESULTS
Results are calculated by linear regression. If a different method than linear regression is used the method must be validated. Control the curve fitting by using suPARnostic® TurbiLatex Controls and undertake corrective actions if results exceed upper and lower limit.

LIMITATIONS OF TEST
Clinical prognostication should not be based on the result of the suPARnostic® TurbiLatex test alone. Interpretation of results must be made considering the patient's clinical history and results of diagnostic tests if available.

EXPECTED VALUES
All individuals have a measurable suPAR level, and in healthy blood donors (N=9305) the suPAR level is around 2 - 3 ng/ml (25-75% interval from 1.76 – 3.23 ng/ml)[1], in patients attending emergency departments around 3 - 6 ng/ml [2],[3] and in patients with severe disease and organ failure, suPAR is often in the double-digits[4],[5]. The higher the level, the higher the risk of disease progression and the worse the prognosis.

ASSAY PROCEDURE
The method for Roche Cobas® c111 analyser should be prepared according the application parameters listed below. When the method is created correctly the machine will perform the analysis automatically after ordering the test.

Application parameters for the Roche Cobas® c111 analyser:

Sample Type	Plasma
Sample volume	10 µL
R1 volume	150 µL
R2 volume	50 µL
Incubation time with R1	p3-p18
Incubation start time with R2	p18
Calculation points	p20, p35
Delay time	0s
Method	2Point End
Kind of process	Linear
Reaction direction	Increasing
Wavelength	583 nm

PERFORMANCE CHARACTERISTICS
Results presented below were obtained with use of suPARnostic® TurbiLatex Reagents on Roche Cobas® c111 analyzer.

LIMIT OF BLANK (LOB)
Three samples, human plasma diluted in 1:16 ratio with water, measured 20 times. In total 60 blank measurements for 1 day.

LIMIT OF DETECTION (LOD)
Limit of detection is the lowest possible detection of suPAR that is not a blank sample.
Five low concentration samples tested in 12 replicates for 2 days.

LIMIT OF QUANTIFICATION (LOQ)
LOQ=LOD

	LOB	LOD	LOQ
Result	1.0 ng/mL	1.5 ng/mL	1.5 ng/mL

LOB, LOD and LOQ were established with accordance to CLSI EP17.

INTERFERENCE
Samples with abnormally elevated levels of hemoglobin, lipids or bilirubin may interfere with assay performance and sensitivity. Rheumatoid factor interferes with suPAR results in approx. 5% of patients.
The substances listed below were tested for interference with the suPARnostic® TurbiLatex Reagents.
Reference samples (Ref. 1 ~3.0 ng/mL and Ref. 6 ~ 17.4 ng/mL) were tested in three replicates with addition of interfering substance. The results were compared to Control sample (Ref. 1/ Ref. 6 with 0.9% saline). No interference was observed below these concentrations:

Substance	Concentration
Bilirubin	87.5 mg/L
Hemoglobin	1.6 g/L
Triglycerides	3.3 g/L

LINEARITY

The suPARnostic® TurbiLatex test is linear from 1.5 ng/mL to 27.7 ng/mL

HOOK EFFECT

The suPARnostic® TurbiLatex shows no prozone effect in concentrations below 65 ng/mL (this is the highest tested suPAR concentration).

REPEATABILITY AND INTERMEDIATE PRECISION

Low, medium and high samples were measured with two replicates in two separate runs per day for 20 days.

Repeatability	Mean	Within run CV	Between runs CV	Total
Low	2.7 ng/mL	8%	4%	8%
Medium	6.0 ng/mL	4%	2%	5%
High	9.9 ng/mL	4%	3%	4%

4 Replicates pr. day, for 20 days.

Factors which were changed during Intermediate precision study:

- Operators (3 different)
- Reagent Lots (3 batches)
- Calibrations (with 2 different Calibrators batch)
- Days (20)

Inter-mediate precision	Mean	Within run CV	Between runs CV	Total
Low	2.9 ng/mL	4%	6%	7%
Medium	5.7 ng/mL	3%	5%	5%
High	9.8 ng/mL	2%	4%	4%

4 Replicates pr. day, for 20 days.

ACCURACY (METHOD COMPARISON)

Bias and correlation calculations toward suPARnostic® ELISA have been performed to estimate the TurbiLatex ability to quantify suPAR in patient samples. Forty samples were measured with three batches of Turbidimetry reagents, results for each batch were compared to suPARnostic® ELISA results. Results below are calculated from all 120 results.

Results:

Sample Type	No. of pairs	Slope	y-Intercept	Pearson correl.	Range value
Plasma	120	0.9497	0.4119	0.915	2.1-13.4 ng/mL

X = suPARnostic® ELISA Y = suPARnostic® TurbiLatex

WASTE HANDLING

Discard unused reagents and waste in accordance with country, federal, state, and local regulations.

REFERENCES

1. Haastrup E, Grau K, Eugen-Olsen J, Thorball C, Kessing LV, Ullum H: Soluble urokinase plasminogen activator receptor as a marker for use of antidepressants. PLoS One 2014, e110555.

2. Raggam RB, Wagner J, Pruller F, Grisold A, Leitner E, Zollner-Schwetz I, et al: Soluble urokinase plasminogen activator receptor predicts mortality in patients with systemic inflammatory response syndrome. J Intern Med 2014, 276(6):651-8.

3. Rasmussen LJ, Ladelund S, Haupt TH, Ellekilde G, Poulsen JH, Iversen K, Eugen-Olsen J, Andersen O. Soluble urokinase plasminogen activator receptor (suPAR) in acute care: a strong marker of disease presence and severity, readmission and mortality. A retrospective cohort study. Emerg Med J. 2016 33(11):769-775.

4. Koch A, Zimmermann HW, Gassler N, Jochum C, Weiskirchen R, Bruensing J, et al.: Clinical relevance and cellular source of elevated soluble urokinase plasminogen activator receptor (suPAR) in acute liver failure. Liver Int 2014;34:1330–1339.

5. Donadello K, Scolletta S, Taccone FS, Covajes C, Santonocito C, Cortes DO, et al: Soluble urokinase-type plasminogen activator receptor as a prognostic biomarker in critically ill patients. J Crit Care. 2014 Feb;29(1):144-9.

For Professional Use		
REF	Σ	
Catalogue no.	Contains sufficient for <n> tests	Use by
IVD	°C °C	LOT
In vitro diagnostic medical device	Temperature Limits	LOT no. (Batch No.)