suPARnostic®

Enzyme immunoassay for quantitative determination of soluble urokinase plasminogen activator receptor in human plasma and serum

Code no. E001 suPARnostic® AUTO Flex ELISA

For other languages visit our web-site:
http://www.virogates.com/

ViroGates

CE IVD

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Monitoring Global Health

Please check www.virogates.com for the most recent updates on all suPARnostic® products, assay instructions as well as other tools.

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INTENDED USE

For *In Vitro* diagnostic use.

The suPARnostic® AUTO Flex ELISA is used for the quantitative determination of soluble urokinase plasminogen activator receptor (suPAR) in human plasma and serum.
Interpretation of results must be made considering the patient's clinical history and if available results of other diagnostic tests.

The suPARnostic® AUTO Flex ELISA with 8 x 12 break-apart wells is allowing flexibility in the number of samples tested (up to 91 samples in single wells) and/or multiple assays to be performed from one set of reagents.

The suPARnostic® AUTO Flex ELISA is designed to run on various automated ELISA platforms.

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 measurable in all bodily fluids. suPAR is a non-specific biomarker which is increased during development [1,2] or presence of disease [3]. The higher the suPAR level, the higher the risk of disease progression and the worse the patient's prognosis.

PRINCIPLES OF ASSAY PROCEDURE

suPARnostic® is a CE/IVD marked product range applied for determination of soluble urokinase Plasminogen Activator Receptor (suPAR) in human EDTA-, heparin-plasma or serum.

The suPARnostic® AUTO Flex ELISA is a simplified double monoclonal antibody sandwich assay where samples and peroxidase-conjugated anti-suPAR are mixed in the mixing plate included prior to incubation in anti-suPAR precoated optically clear microwells. The assay utilizes monoclonal mouse and rat antibodies against human suPAR. The suPAR standard is calibrated against an internal Golden Standard and all values are calculated back to this to ensure samples from different labs and/or different assay lots can be directly compared when the suPARnostic® AUTO Flex ELISA is used. suPAR concentrations determined using the suPARnostic® AUTO Flex ELISA are expressed as ng/mL.

In the assay, the suPAR standards, curve control and patient specimens are mixed with peroxidase-conjugated anti-suPAR in the white microwell mixing plate included. This solution is then transferred from the white to the optically-clear microwell plate which is pre-coated with anti-suPAR antibody. During a one-hour incubation period, a sandwich is formed consisting of solid-phase antibody, suPAR and the peroxidase-conjugated antibody. Following a washing step, where unbound material is removed, a chromo- genic substrate is added to the wells. The more suPAR a sample contains, the more intense is the blue color which develops. After 20 minutes incubation in the dark, the color development is stopped by the addition of sulphuric acid which changes the color in the wells to yellow. The absorbance at 450 nm is measured using a microtiter plate reader. A calibration curve is prepared from the suPAR standard, and the con- centration of suPAR in the patient specimen is determined by interpolation.
Results above the highest standard should be diluted and measured again to get accurate concentration.

REAGENTS

A. Materials supplied in suPARnostic® AUTO Flex ELISA **(Code No. E001)**
(Procedure for automated ELISA processors can be found on page 22)

This kit contains reagents sufficient to perform 96 tests - up to 91 patient samples in single wells. The 'break-apart' wells give the flexibility to perform as many or few tests as required. 3 Standards, Blank, and Curve Control must be included with each assay run. Sufficient wells should be removed from the plate for the number of samples to be tested and the remainder returned to the storage pouch with desiccant and stored at 4°C until required for the next analysis.

1. **White Microtiter Plate** with 96 wells for mixing samples, standards, control and samples with peroxidase-conjugate, in a clear plastic pouch. Quantity: 1 plate. Preparation: Ready to use.

2. **Clear Microtiter Plate** with break-apart wells precoated with anti-suPAR antibody. There are 96 test wells per plate, in an aluminium storage pouch with desiccant sachet. Quantity: 8 wells x 12 strips. Preparation: Ready to use.

3. **Standards** Recombinant suPAR (soluble urokinase plasminogen activator receptor), in PBS buffer with proprietary additives and 0.05% Bronidox® as preservative. Quantity: 5 Standards, each vial contains 600 µL of standard containing protein stabilizer. For suPAR concentration in this kit lot, please see separate Analytical Value Sheet.

4. **Curve Control** Recombinant suPAR, in PBS buffer with proprietary additives and 0.05% Bronidox®. Quantity: 1 vial with 600 µL.

5. **Peroxidase Conjugate** (200 x concentrated). Peroxidase-conjugated mouse anti-human suPAR in buffer (containing 50% Ethylene-glycol) with proprietary additives and antimicrobial agent. Quantity: 1 brown vial with 200 µL. Preparation: Depending on the number of wells to be used, prepare the required amount in a suitable container and use within 30 minutes. **Precaution:** Light sensitive, avoid unnecessary exposure to light. Ensure that conjugate mix is used within 30 minutes of preparation.

6. **Dilution Buffer** PBS buffer, pH 7.4, with proprietary additives and 0.05% Bronidox® as preservative. Quantity: 2 x 18 mL. Preparation: Ready to use.

7. **TMB Solution** 3, 3', 5, 5'-tetramethylbenzidine (TMB). Precaution: Light sensitive, avoid unnecessary exposure to light. Quantity: 2 x 11 mL. Ready to use. Remove only the amount required for each assay (with 10% extra for pipetting margin).

8. **Wash Buffer** 10 x concentration of PBS buffer with proprietary additives and 0.05% Bronidox® as preservative. Quantity: 1 bottle containing 100 mL. Preparation: Dilute one plus nine (1:10) with distilled or deionized water.

9. **Stop Solution** 0.45 M suphuric acid (H2SO4). Quantity: 16 mL. Preparation: Ready to use.

10. **Sealing Tape** Quantity 6 Sheets of adhesive tape.

11. **Empty Plastic Bottle** For preparation of conjugate solution, if required. Quantity: 1 bottle. Ready to use.

B. Materials required but not provided

- Adjustable pipette with tips, 10 µL – 100 µL, 100 µL – 1000 µL
- Multi-channel precision pipette, 50 - 300 µL adjustable
- Reagent reservoirs and/or small tubes for preparing dilutions
- Timer
- Deionized or distilled water
- Microtiter plate reader capable of reading absorbency at 450 nm and an additional reference filter 650 nm for dual wavelength reading (450 – 650 nm)
- ELISA microplate washer, squeeze bottle or suitable container to wash wells
- Absorbent paper or cloth
- Refrigerator

C. Reagent storage and stability

Store kit components at 2-8°C.Expiry is indicated on the labels.

REAGENT PRECAUTIONS AND RECOMMENDATIONS

- For professional users.
- Do not use kit components beyond the indicated kit expiration date.
- Do not mix reagents from different kit lots.
- Stop Solution – component number 9 – contains 0.45 M sulphuric acid; avoid contact with skin and eyes.
- Do not expose reagents to excessive light.
- Do not freeze any of the kit components.
- Use only the microtiter wells provided with the kit.
- 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 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. All solutions supplied should be handled carefully and disposed of in accordance with national and local regulations.

SAMPLE COLLECTION AND STORAGE

To prepare plasma samples, whole blood is drawn into a centrifuge tube containing EDTA or heparin anti-coagulant. Centrifuge the blood at 3,000 x g for 10 minutes. Serum samples are prepared according to the recommendation from the manufacturer of the blood collection tubes.
Transfer and store samples in separate, marked tubes. Date and identify each sample. For long-term storage, keep at -20°C. Avoid freeze/thaw cycles.

Grossly hemolyzed, lipemic or microbiologically contaminated samples should not be used. Samples with abnormally elevated levels of hemoglobin or bilirubin may interfere with assay performance and sensitivity.

Be aware of possible dilution of suPAR in the case of transfusion, infusion or similar.

Sample Type	*Sample Requirement*
Plasma or Serum	*Duplicate: 25* µL; *Singlets*: 15 µL

ASSAY PROCEDURE 1 - Full Plate (Duplicate)

suPARnostic® AUTO Flex ELISA **(Code No. E001)**

Equilibrate all reagents to room temperature (18 - 26°C) for 1 hour prior to use.
Preparation of buffers prior to use:

1. Wash Buffer, working solution

Dilute the stock solution one plus nine parts (1:10) with distilled or deionized water. The whole bottle should be added to 900 mL distilled or deionized water. The working solution may be stored at 2 – 8 °C for up to 6 months.

2. Peroxidase Conjugate, working solution

Prepare the Conjugate in the empty plastic bottle provided. Dilute 65 µL of the stock solution in 13 mL of the Dilution Buffer (Component 6). The Conjugate should be protected from light and used within 30 minutes of preparation.

Procedure

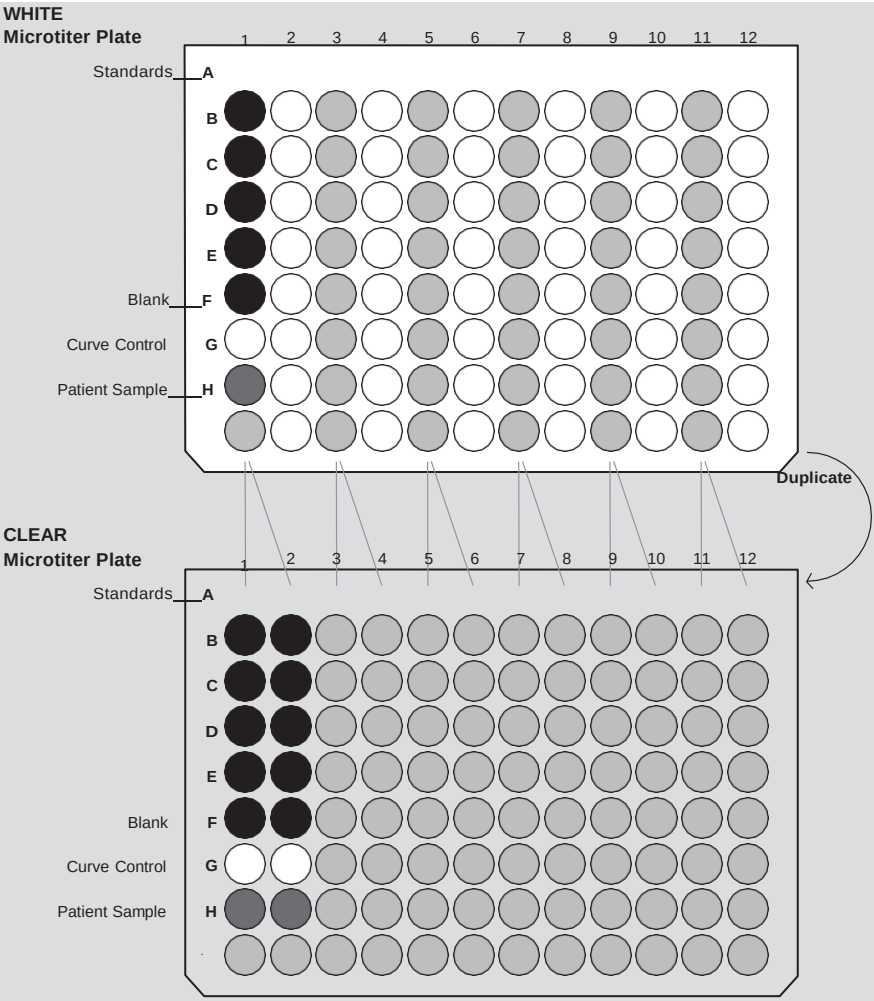
1. Transfer a 25 µL aliquot from each suPAR Standard vial (3a to 3e), to the columns A1 – E1, transfer 25 µL Dilution Buffer (Component 6) to well F1 for the Blank and 25 µL of the Curve Control (blue cap) to well G1 in the White mixing plate.

2. Transfer a 25 µL aliquot of each sample to a well in the White mixing plate (wells H1, A3 – H3, A5 – H5, etc).

3. Pipette 225 µL of the Peroxidase Conjugate solution (prepared above) into each of the wells in the White mixing plate using a multi-channel pipette.

4. Mix gently by slowly pipetting the contents of each well four times up and down in the pipette tip. Transfer, in duplicate, 100 µL aliquots of the mixed contents from each well in the White mixing plate into wells of the Clear coated plate using a multi-channel pipette.

Exchange the pipette tips after mixing and transfer in duplicate of each column. E.g. from the Standards, Blank and Curve Control, and sample in column 1 of the White mixing plate, transfer 100 µL to column (A-H) 1 and 100 µL to column (A-H) 2 in the Clear coated plate. Similarly mix and transfer the samples in the White mixing plate column 3 to the wells in column 3 and 4 of the Clear coated plate etc.



5. Cover the Clear plate with Sealing Tape to prevent evaporation, and incubate for 1 hour at room temperature (18 - 26°C) in the dark.

6. Remove the Sealing Tape and decant the contents from the wells.

7. Wash wells five times with 250 µl per well of prepared Wash Buffer. This may be done with a multi-channel pipette, or by gently pouring the Wash Buffer into the wells. After emptying the contents of the wells, pipette 250 µL 1X Wash Buffer into the wells and repeat the process a further four times. Tap plate gently between each wash step. Carefully blot plate onto absorbent paper after the final wash and ensure no bubbles remain in the wells.

Note: Improper washing will give erroneous results. Do not allow the wells to dry out between incubations.

8. Add 100 µL TMB Solution to each well, cover the Clear coated plate with Sealing Tape and incubate for 20 minutes at room temperature (18 - 26°C) in the dark.

9 Remove the Sealing Tape and stop the reaction by adding 100 µL Stopping Solution to each well. The colour should change from blue to yellow due to the pH change.

10. Read the absorbance at 450 nm within 30 minutes of stopping the reaction.

Note: For dual wavelength readers, use a reference filter at approximately 650 nm. Ensure there are no air bubbles in any of the wells.

Summary of Assay Procedure – Full Plate (Duplicate)

The White mixing plate is used in all the following steps:

Add Standards, Curve Control, Blank and samples	25 µL/well
All wells: Add Conjugation working solution* and mix	225 µL/well
All wells: Transfer diluted samples from White mixing plate to Clear coated plate *	100 µL/well

** Use an 8-channel pipette*

The Clear coated plate is used in all the following steps:

Incubate at room temperature (18 - 26°C) in the dark	1 hour
Empty the plate and Wash (250 µL/well)	5 cycles
Add TMB Solution *	100 µL/well
Incubate at room temperature (18-26°C) in the dark	20 minutes
Add Stop Solution *	100 µL/well
Read absorbance	450 nm
Reference filter, optional	650 nm

** Use an 8-channel pipette*

CALCULATION OF RESULTS

ViroGates has developed a program for calculation of suPAR values from ABS 450nm values. The program is free and can be downloaded at www.virogates.com or requested by e-mail (info@virogates.com).

Alternatively, calculate the corrected absorbance by subtracting the absorbance of the Blank (0 ng/mL). Construct a standard curve in a linear system of co-ordinates by plotting the corrected absorbance of the Standards (ordinate, y-axis) against the corresponding suPAR concentration (abscissa, x-axis). Draw the best-fitting line (see Figure 1).

Determine the suPAR concentration of the Curve Control and of each patient specimen by interpolation on the curve. Patient specimens giving absorbance values above the standard should be pre-diluted 1 + 2 with Dilution Buffer (Component 6) in a test tube (not provided) and then retested according to the assay procedure. After assaying, multiply the result by 3 (the additional dilution factor).

Curve control

The suPAR concentration determined for the Curve Control should fall within the range stated in the separate Analytical Value Sheet. If this is not the case, further investigation should be made to ascertain whether the cause is imprecise technique, improper handling or reagent deterioration.

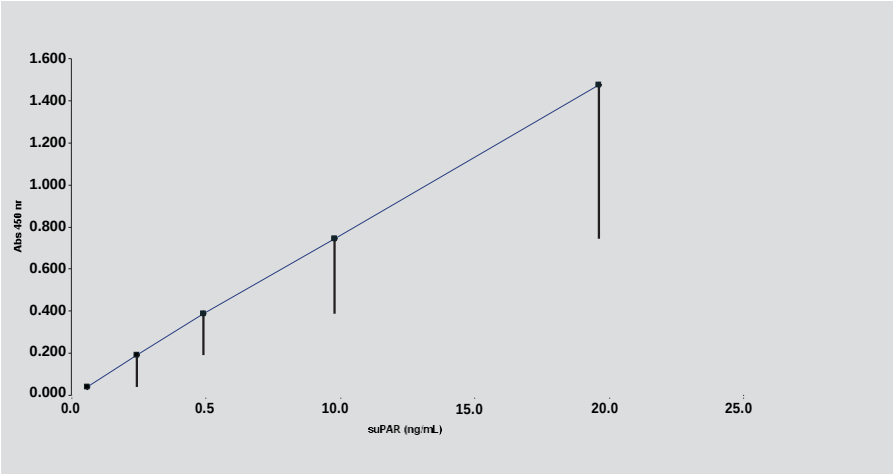


Figure 1: Typical standard curve

PERFORMANCE CHARACTERISTICS

A. Imprecision

For each of five plasma samples the imprecision was calculated from the means of up to eight duplicate determinations for up to five separate runs. Standard deviations and coefficients of variation (CV) are listed in the table below.

Sample (ng/mL)	Within days CV	Between days CV	Total CV	n
2.3	3.5%	5.1%	6.0%	40
2.4	4.7%	3.5%	5.6%	40
3.7	1.3%	2.3%	2.4%	14
5.4	2.1%	2.2%	2.9%	16
7.2	1.7%	1.7%	2.3%	16

B. Recovery

Spiked samples were prepared by adding varying amounts of suPAR to three plasma specimens.

Sample 1 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	-	-	-	
	17.1	13.7	80%	
	12.1	10.2	84%	
2.1	7.1	5.4	76%	89%
	4.1	3.9	95%	
	3.1	2.9	94%	
	2.7	2.5	93%	
	2.1	2.1	100%	

Sample 2 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	22.0	19.9	90%	
	17.0	14.9	88%	
	12.0	10.5	88%	
2.0	7.0	6.2	89%	94%
	4.0	4.0	100%	
	3.0	3.0	100%	
	2.6	2.6	100%	
	2.0	2.0	100%	

Sample 3 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	-	-	-	
	19.0	16.7	88%	
	14.0	12.4	89%	
4.0	9.0	8.0	89%	95%
	6.0	5.8	97%	
	5.0	5.1	102%	
	4.6	4.6	100%	
	4.0	4.0	100%	

C. Dilution / Linearity

Three plasma specimens were diluted with Dilution Buffer and assayed after dilution. The neat sample is set to 100%. The results are summarized in the table below.

Sample A	Expected ng/mL	Found ng/mL	% found	Mean
neat	15.6	15.6	100%	
80%	12.4	13.3	107%	
60%	9.3	9.4	101%	102%
50%	7.8	8.2	105%	
40%	6.2	6.3	102%	
30%	4.7	4.6	98%	

Sample B	Expected ng/mL	Found ng/mL	% found	Mean
neat	10.5	10.5	100%	
80%	8.4	8.9	106%	
60%	6.3	6.6	105%	102%
50%	5.3	5.4	102%	
40%	4.2	4.3	102%	
30%	3.2	3.1	97%	

Sample C	Expected ng/mL	Found ng/mL	% found	Mean
neat	6.8	6.8	100%	
80%	5.4	5.5	102%	
60%	4.1	4.2	102%	97%
50%	3.4	3.3	97%	
40%	2.7	2.5	93%	
30%	2.0	1.8	90%	

D. Detection limit

By determination of the mean absorbance + 3 SD of the Blank (0 ng/µL), the detection limit of the assay was estimated to be 0.1 ng/mL.

ASSAY PROCEDURE 2 - Strip Method (Singlets)

suPARnostic® AUTO Flex ELISA **(Code No. E001)**

Procedure 2 is for users running less than a full plate at a time. Equilibrate all reagents to room temperature (18 - 26°C) for 1 hour prior to use. Determine the required number of wells from both the White mixing plate and the Clear coated plate. Take the White mixing plate from the zip-lock bag. After use, dry and return the White mixing plate to the zip-lock bag for future use. Carefully cut the end of the foil pouch and remove the Clear coated plate from the pouch; return unused wells to the foil pouch and return to 4°C storage for future use. Note: Remember to retain the White mixing plate and the plate frame for future use!

Preparation of buffers prior to use:

1. **Wash Buffer, working solution**
   Dilute the required amount of stock solution one plus nine parts (1:10) with distilled or deionized water. For the entire plate, the whole bottle should be added to 900 mL distilled or deionized water. For each strip to be used a minimum of 10 mL Wash Buffer should be prepared. More washing solution will be required, if an automatic plate washer is used. The working solution may be stored at 2 – 8°C for up to 6 months.

2. **Peroxidase Conjugate, working solution**
   Prepare the required amount of Conjugate in a clean plastic container of suitable size as described in the table below (using Dilution Buffer, Component 6). For less than three strips it is recommended the Conjugate be added using a single channel pipette. For three or more strips the Conjugate may be added using a multi-channel pipette. The Conjugate should be used within 30 minutes of preparation. Unused Conjugate stock should be protected from light and returned to 4°C storage as soon as possible.

Number of strips	Volume of Conjugate stock (µL)	Volume of Dilution Buffer (mL)
1	15	3
2	20	4
3	25	5
4	30	6
5	35	7
6	40	8
7	45	9
8	50	10
9	55	11
10	60	12
11	65	13
12	70	14

Procedure

1. Decide on the number of Standards to be used from 3-5 Standards.

Note: This procedure is based on 3 Standards.

2. Calculate the number of wells required for the assay and mark them on the White mixing plate; consider covering wells not to be used, to avoid sample contamination. After use, dry the White mixing plate and return to the zip-lock bag for future use.

3. Calculate the number of strips required for the assay. Carefully cut open the foil pouch and take out the plate. Remove extra strips from the Clear coated plate and return those unused strips to the foil pouch with desiccant, seal and return to 4°C storage for future use.

Note: On completion of the assay retain both the plate and frames for future use, if less than the full plate has been used.

4. Transfer 15 µL of the Standards (clear cap, 3a, 3c and 3e) to well A1 – C1 in the White mixing plate.

5. Transfer 15 µL of Dilution Buffer (Component 6) to well D1 in the White mixing plate (Blank).

6. Transfer 15µL of the Curve Control (blue cap) to well E1 in the White mixing plate.

7. Transfer 15 µL of each sample to subsequent wells in the White mixing plate as required.

Note: 3 Standards, Blank and Curve Control must be included with each assay.

8. Pipette 135 µL of the Peroxidase Conjugate solution (prepared above) to each used well. For less than three strips it is recommended this be done using a single channel pipette. For three or more strips an 8-channel multi-pipette and suitable reagent reservoir may be used.

9. Mix gently by slowly pipetting the contents of each well a few times up and down in the pipette tip, then transfer 100 µL to the corresponding well in the Clear coated plate. This can be done using a multi-channel pipette. Ensure the pipette tips are changed between each addition.

10. Cover the Clear coated plate with Sealing Tape (this may be cut to the correct size prior to use) to prevent evaporation and incubate for 1 hour at room temperature (18 - 26°C) in the dark.

11. Remove the Sealing Tape and decant the contents from the wells.

12. Wash wells five times with 250 µl per well of prepared Wash Buffer. This may be done using a multi- or single channel pipette. After emptying the contents of the wells, pipette 250 µL 1X Wash Buffer into the wells and repeat the process a further four times. Alternatively, the Wash Buffer may be gently poured into the wells or added using a squeeze bottle. Tap plate gently onto absorbent paper between each wash step. Carefully blot plate after the final wash and ensure no bubbles remain in the wells.

Note: Improper washing will give erroneous results. This step must be carried out carefully. Do not allow the wells to dry out between incubations.

13 Add 100 µL TMB Solution to each well, cover the Clear coated plate with Sealing Tape and incubate for 20 minutes at room temperature (18 - 26°C) in the dark.

Note: TMB Solution is easily contaminated. Only remove the required amount for the assay from the bottle (including 10% extra for pipetting margin). Discard unused TMB Solution. Do not return to bottle.

14 Remove the Sealing Tape and stop the reaction by adding 100 µL Stop Solution to each well. The colour should change from blue to yellow due to the pH change.

15 Read the absorbance at 450 nm within 30 minutes of stopping the reaction.

Note: For dual wavelength readers use a reference filter at approximately 650 nm. Ensure there are no air bubbles in any of the wells.

Summary of Assay Procedure – Strip Method (Singlets)

The White mixing plate is used in all the following steps:

Add Standards, Curve Control, Blank and samples	15 µL/well
All wells: Add Conjugation working solution* and mix	135 µL/well
All wells: Transfer diluted samples from White mixing plate to Clear coated plate *	100 µL/well

The Clear coated wells are used in all the following steps:

Incubate at room temperature (18 - 26°C) in the dark	1 hour
Empty the plate and wash (250µL/well)	5 cycles
Add TMB Solution *	100 µL/well
Incubate at room temperature (18 – 26°C) in the dark	20 minutes
Add Stop Solution *	100 µL/well
Read absorbance	450 nm
Reference filter, optional	650 nm

** Use an 8-channel pipette if more than 3 strips are used*

CALCULATION OF RESULTS

ViroGates has developed a program for calculation of suPAR values from ABS 450nm values. The program is free and can be downloaded at www.virogates.com or requested by e-mail (info@virogates.com).

Alternatively, calculate the corrected absorbance by subtracting the absorbance of the Blank (0 ng/mL) from all wells in the assay. Construct a standard curve in a linear system of co-ordinates by plotting the corrected absorbance of the Standard (ordinate, y-axis) against the corresponding suPAR concentration (abscissa, x-axis). Draw the best fitting line (see Figure 2)

Determine the suPAR concentration of the Curve Control and of each patient specimen by interpolation on the curve. Patient specimens giving absorbance values above the highest standard should be pre-diluted 1 + 2 with Dilution Buffer (Component 6) in a test tube (not provided) and then retested according to the assay procedure. After assaying, multiply the result by 3 (the additional dilution factor).

Curve control

The suPAR concentration determined for the Curve Control should fall within the range stated in the separate Analytical Value Sheet. If not, further investigation should be made to ascertain whether the cause is imprecise technique, improper handling or reagent deterioration.

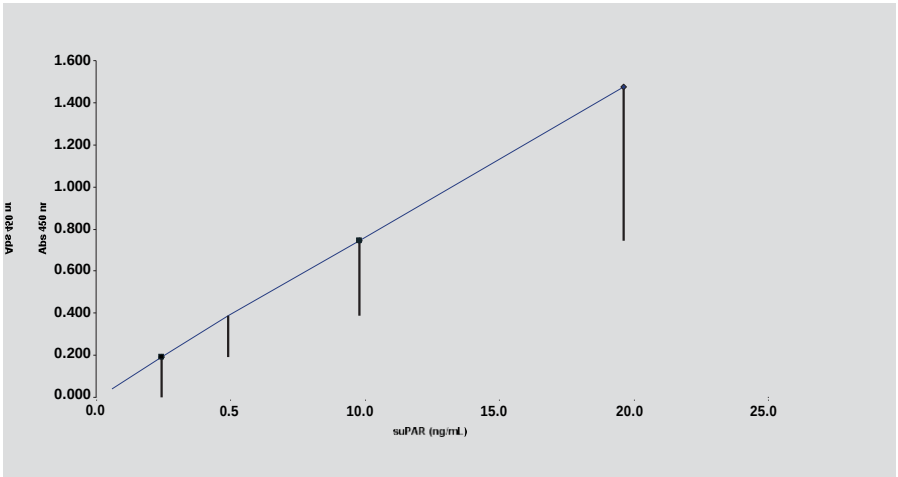


Figure 2: Typical standard curve

PERFORMANCE CHARACTERISTICS

A. Imprecision

For each of five plasma samples the imprecision was calculated from the means of up to eight duplicate determinations for up to five separate runs. Standard deviations and coefficients of variation (CV) are listed in the table below. These results were done using one Standard.

Sample (ng/mL)	Within days CV	Between days CV	Total CV	n
2.3	3.5%	5.1%	6.0%	40
2.4	4.7%	3.5%	5.6%	40
3.7	1.3%	2.3%	2.4%	14
5.4	2.1%	2.2%	2.9%	16
7.2	1.7%	1.7%	2.3%	16

B. Recovery

Spiked samples were prepared by adding varying amounts of suPAR to three plasma specimens. These results are made using only one Standard.

Sample 1 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	22.12	19.15	87%	
	17.12	14.21	83%	
	12.12	10.67	88%	
2.12	7.12	5.30	74%	87%
	4.12	3.92	95%	
	3.12	2.85	91%	
	2.72	2.51	92%	
	2.12	2.12		

Sample 2 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	21.97	20.17	92%	
	16.97	15.22	90%	
	11.97	11.20	94%	
1.97	6.97	6.52	94%	96%
	3.97	3.89	98%	
	2.97	3.12	105%	
	2.57	2.66	104%	
	1.97	1.97		

Sample 3 ng/mL	Expected ng/mL	Found ng/mL	Recovery	Mean
	24.15	21.43	89%	
	19.15	16.47	86%	
	14.15	12.14	86%	
4.15	9.15	8.35	91%	93%
	6.15	6.13	100%	
	5.15	5.18	101%	
	4.75	4.83	102%	
	4.15	4.25		

C. Dilution / Linearity

Three plasma specimens were diluted with Dilution Buffer and assayed after dilution. The neat sample is set to 100%. The results are summarized in the table below.

Sample A	Expected ng/mL	Found ng/mL	% found	Mean
neat	15.3	15.1	99%	
80%	12.2	13.6	111%	
60%	9.2	9.0	98%	102%
50%	7.6	7.9	103%	
40%	6.1	6.4	104%	
30%	4.6	4.5	98%	

Sample B	Expected ng/mL	Found ng/mL	% found	Mean
neat	10.4	10.7	103%	
80%	8.3	8.6	104%	
60%	6.2	6.7	107%	103%
50%	5.2	5.7	109%	
40%	4.2	4.4	107%	
30%	3.1	2.8	89%	

Sample C	Expected ng/mL	Found ng/mL	% found	Mean
neat	6.5	6.9	106%	
80%	5.2	5.5	105%	
60%	3.9	4.0	103%	98%
50%	3.3	3.3	100%	
40%	2.6	2.3	88%	
30%	2.0	1.7	87%	

D. Detection limit

By determination of the mean absorbance + 3 SD of the Blank (0 ng/µL), the detection limit of the assay was estimated to be 0.1 ng/mL.

ASSAY PROCEDURE 3 - Automated ELISA

suPARnostic ® AUTO Flex ELISA **(Code No. E001)**

Procedure 3 is for users using an automated ELISA processor to perform the assay. The suPARnostic® AUTO Flex ELISA is validated to be used with several commercially available devices like BEP2000 processors and similar. ViroGates are continuously adding protocols for automated ELISA processors.

For valid runs on the BEP2000 and other processors only use the programs recommended by ViroGates. These can be ordered by e-mail (info@virogates.com).

The following procedure is based on BEP2000 processors.

1. Aspirate 325 µL Peroxidase Conjugate and then aspirate additionally to that volume 35 µL of Std A.
2. Dispense 350 µL into the mixing plate. Use same procedure with the remaining Standards, Blank, Curve Control as well as the Samples.
3. Aspirate 210 µL of Std A and dispense 100 µL to A1-B1 in the pre-coated clear plate. (Begin with Std A and repeat for following Standards, Blank, Curve Control and Samples.)
4. Incubate for 1 hour.
5. Perform 3 x 250 µL wash cycles using suPARnostic wash buffer finishing with aspire sweep. Perform an extra cycle of aspire sweep.
6. Add 100 µL of TMB Solution into all the wells followed by 5 sec. plate shake.
7. Incubate 20 min.
8. Stop the reaction by adding 100 µL of Stop Solution to all the wells.
9. Read the plate using 450 nm wavelength.

CALCULATION OF RESULTS

Automated ELISA processors are able to calculate the results automatically through the delivered software.

If this is not an option please see page 18 for manual calculation.

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1. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. Eugen-Olsen J, Andersen O, Linneberg A, Ladelund S, Hansen TW, Langkilde A, Petersen J, Pielak T, Møller LN, Jeppesen J, Lyngbaek S, Fenger M, Olsen MH, Hildebrandt PR, Borch-Johnsen K, Jørgensen T, Haugaard SB. J Intern Med. 2010 Sep;268(3):296-308.

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