

Human (VASH1)

ELISA Kit Instruction

Catalogue No.

201-12-5231

Preface

Please carefully read this instruction before using. This ELISA kit is based on the principle of double-antibody sandwich technique to detect Human (VASH1). Be used only for research purposes, not be used for medical diagnosis.

Full Name

Human Vasohibin 1 (VASH1) ELISA Kit

Intended Use

This kit is used to assay the Vasohibin 1(VASH1) in the sample of human's serum, blood plasma, and other related tissue Liquid.

Test principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Vasohibin 1(VASH1) in samples. Add Vasohibin 1(VASH1) to monoclonal antibody Enzyme well which is pre-coated with Human Vasohibin 1(VASH1) monoclonal antibody, incubation; then, add



Vasohibin 1 (VASH1) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance Vasohibin 1 (VASH1) of sample were positively correlated.

Materials supplied in the Test Kit

1	Standard (7200pg/ml)	0.5ml
2	Standard diluent	3m1
3	Microelisa Strip plate	12well×8strips
4	Str- HRP-Conjugate Reagent	6m1
5	30×wash solution	20m1
6	Biotin- VASH1 Ab	1ml
7	Chromogen Solution A	6m1
8	Chromogen Solution B	6m1
9	Stop Solution	6m1
10	Instruction	1
11	Closure plate membrane	2
12	Sealed bags	1

Materials required but not supplied

1. 37 °C incubator

English Instruction enzyme-linked immunosorbent assay

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2. Standard Enzyme reader

3. Precision pipettes and Disposable pipette tips

4. Distilled water

5. Disposable tubes for sample dilution

6. Absorbent paper

Important Notes

1. Been taken out from the 2-8°C environment, the kit should be balanced

30 minutes in the ambient temperature then use. If the Coated plates of

Enzyme haven't been used up after opened, the remaining plates should be

stored in Sealed bag.

2. For each step, add Sample with sample injector which should be calibrated

frequently, in order to avoid unnecessary experimental tolerance.

3. The operation shall be carried out accordance to the instructions

strictly. And test results must be based on the readings of the Enzyme

reader.

4. In order to avoid cross-contamination, it is forbidden to re-use the

suction head and seal plate membrane in your hands.

5. All samples, washing buffer and each kind of reject should according

to infective material process.

6. The idle agents shall be put up or covered. Do not use reagent with

different batches. And use them before expired date.

7. The substrate B is light-sensitive. Prolonged exposure to light is

forbidden.

Washing method

WEB: www. sunredbio.com



<u>Manually washing method</u>: shake away the remain liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

<u>Automatic washing method:</u> if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

Specimen requirements

- 1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active
- 2. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20 °C to preserve, Avoid repeated freeze-thaw cycles.
- 3. <u>serum</u>—coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 4. <u>plasma-use</u> suited EDTA or citrate plasma as an anticoagulant, mix 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 5. <u>Urine-collect</u> sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.



- 6. <u>cell culture supernatant</u>—detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, detect the composition of cells, Dilute cell suspension with PBS (PH7. 2-7. 4), Cell concentration reached 1 million / ml, repeated freeze—thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 7. <u>Tissue samples</u>— After cutting samples, check the weight, add PBS (PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting, add PBS (PH7.4), Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Assay procedure

1. Standard dilution:

this test kit will supply one original Standard reagent, please dilute it by yourself according to the instruction.

3600pg/ml	Standard No. 5	120 µ 1 Original Standard + 120 µ 1 Standard diluents
1800pg/ml	Standard No. 4	120 µ 1 Standard No.5 + 120 µ 1 Standard diluents
900pg/ml	Standard No. 3	120 µ 1 Standard No. 4 + 120 µ 1 Standard diluent
450pg/ml	Standard No.2	120 µ 1 Standard No. 3 + 120 µ 1 Standard diluent
225pg/ml	Standard No.1	120 µ 1 Standard No. 2 + 120 µ 1 Standard diluent

2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample shall be made according to your required quantity, and try to use the duplicated well as possible.



3. Inject samples:

- ① Blank well: don't add samples and VASH1 -antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.
- ② Standard wells: add standard $50 \,\mu l$, Streptavidin-HRP $50 \,\mu l$ (since the standard already has combined biotin antibody, it is not necessary to add the antibody);
- ③ To be test wells: add sample $40 \,\mu$ l, and then add both VASH1 -antibody $10 \,\mu$ l and Streptavidin-HRP $50 \,\mu$ l. Then seal the sealing memberance, and gently shaking, incubated 60 minutes at 37 °C.
- 4. Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.
- 5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.
- 6. Add chromogen solution A 50 μ 1, then chromogen solution B 50 μ 1 to each well. Gently mixed, incubate for 10 min at 37°C away from light.
- 7. Stop: Add Stop Solution 50 µl into each well to stop the reaction (the blue changes into yellow immediately).
- 8. Final measurement: Take blank well as zero, measure the optical densit (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.
- 9. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.



Summary procedures

Preparing reagents, samples and standards

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Add prepared samples and standards, antibodies labeled with enzyme, reacting 60

minutes at 37 ℃

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Plate washed five times, adding Chromogen solution A, B, reacting 10 minutes at 37℃

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Add stop solution

J

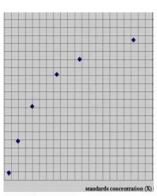
measure the OD value within 10min

1

Calculation

Calculate

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve (the result is the sample density)



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or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density.

TEL:86-21-51877391

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Sensitivity, Assay range

Sensitivity: 22.338pg/ml

(The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.)

Assay range: 25pg/ml→6600pg/ml

Intra-assay Precision: 3 samples with low, middle and high level Human VASH1 were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level Human VASH1 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Package size

96T per box

Validity & Storage

6 months (2-8℃)

12 months (Below-20℃)



Human (CT-1)

ELISA Kit Instruction

Catalogue No.

201-12-1229

Preface

Please carefully read this instruction before using. This ELISA kit is based on the principle of double-antibody sandwich technique to detect Human cardiotrophin 1, CT-1. Be used only for research purposes, not be used for medical diagnosis.

Full Name

Human cardiotrophin 1, CT-1 ELISA Kit

Intended Use

This kit is used to assay the cardiotrophln 1 (CT-1) in the sample of human's serum, blood plasma, and other related tissue Liquid.

Test principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human cardiotrophln 1(CT-1)in samples. Add cardiotrophln 1(CT-1)to monoclonal antibody Enzyme well which is pre-coated with Human cardiotrophln 1(CT-1)monoclonal antibody,



incubation; then, add (CT-1) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance cardiotrophln 1(CT-1) of sample were positively correlated.

Materials supplied in the Test Kit

1	Standard (240ng/L)	0.5ml
2	Standard diluent	3m1
3	Microelisa Stripplate	12well×8strips
4	Str- HRP-Conjugate Reagent	6m1
5	30×wash solution	20m1
6	Biotin- CT-1 Ab	1ml
7	Chromogen Solution A	6m1
8	Chromogen Solution B	6m1
9	Stop Solution	6m1
10	Instruction	1
11	Closure plate membrane	2
12	Sealed bags	1

Materials required but not supplied

- 1. 37 °C incubator
- 2. Standard Enzyme reader



- 3. Precision pipettes and Disposable pipette tips
- 4. Distilled water
- 5. Disposable tubes for sample dilution
- 6. Absorbent paper

Important Notes

- 1. Beening taken out from the 2-8°C environment, the kit should be balanced 30 minutes in the ambient temperature then use. If the Coated plates of Enzyme haven't been used up after opened, the remaining plates should be stored in Sealed bag.
- 2. For each step, add Sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
- 3. he operation shall be carried out accordance to the instructions strictly. And test results must be based on the readings of the Enzyme reader.
- 4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
- 5. All samples, washing buffer and each kind of reject should according to infective material process.
- 6. The idle agents shall be put up or covered. Do not use reagent with different batches. And use them before expired date.
- 7. The substrate B is light-sensitive. Prolonged exposure to light is forbidden.



Washing method

<u>Manually washing method:</u> shake away the remain liquid in the enzyme plates; place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

<u>Automatic washing method:</u> if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

Specimen requirements

- 1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active
- 2. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in -20 °C to preserve, Avoid repeated freeze-thaw cycles.
- 3. <u>serum-</u> coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 4. <u>plasma</u>—use suited EDTA or citrate plasma as an anticoagulant, mix 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 5. <u>Urine-collect</u> sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid



Reference to it.

6. <u>cell culture supernatant</u>—detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, detect the composition of cells, Dilut cell suspension with PBS (PH7. 2-7. 4), Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

7. <u>Tissue samples</u>— After cutting samples, check the weight, add PBS (PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting, add PBS (PH7.4), Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Assay procedure

1. Standard dilution:

this test kit will supply one original Standard reagent, please dilute it by yourself according to the instruction.

120ng/L	Standard No.5	120 µ 1 Original Standard + 120 µ 1 Standard diluents
60ng/L	Standard No.4	120 µ 1 Standard No. 5 + 120 µ 1 Standard diluents
30ng/L	Standard No.3	120 µ 1 Standard No.4 + 120 µ 1 Standard diluent
15ng/L	Standard No.2	120 µ 1 Standard No. 3 + 120 µ 1 Standard diluent
7.5ng/L	Standard No.1	120 µ 1 Standard No. 2 + 120 µ 1 Standard diluent

2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample shall be made according to your required quantity, and try to use the duplicated well as possible.



3. Inject samples:

- ① Blank well: don't add samples and CT-1-antibody labeled with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.
- ② Standard wells: add standard $50 \mu l$, Streptavidin-HRP $50 \mu l$ (since the standard already has combined biotin antibody, it is not necessary to add the antibody);
- ③ To be test wells: add sample $40 \,\mu$ l, and then add both CT-1-antibody $10 \,\mu$ l and Streptavidin-HRP $50 \,\mu$ l. Then seal the sealing memberance, and gently shaking, incubated 60 minutes at 37 °C.
- 4. Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.
- 5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.
- 6. Add chromogen solution A 50 μ 1, then chromogen solution B 50 μ 1 to each well. Gently mixed, incubate for 10 min at 37°C away from light.
- 7. Stop: Add Stop Solution 50 µ 1 into each well to stop the reaction(the blue changes into yellow immediately).
- 8. Final measurement: Take blank well as zero, measure the optical densit (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.
- 9. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.



Summary procedures

Preparing reagents, samples and standards

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Add prepared samples and standards, antibodies labeled with enzyme, reacting 60

minutes at 37 ℃

1

Plate washed five times, adding Chromogen solution A, B, reacting 10 minutes at 37°C

J

Add stop solution

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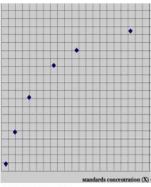
measure the OD value within 10min

1

Calculation

Calculate

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve (the result is the sample density)



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or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density.



Sensitivity, Assay range

Sensitivity: 0.742ng/L(The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.)

Assay range: $0.8 \text{ng/L} \rightarrow 200 \text{ng/L}$

Specificity

This assay has high sensitivity and excellent specificity for detection of CT-1. No significant cross-reactivity or interference between CT-1 and analogues was observed.

- * Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between CT-1 and all the analogues, therefore, cross reaction may still exist.
- * Sample linear regression with the expected concentration of the correlation coefficient R is over 0.95

Intra-assay Precision: 3 samples with low, middle and high level human CT-1 were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level human CT-1 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%

Package size

96T perbox

validity&Storage

six months $(2-8^{\circ})$



Human(ECSM1)

ELISA Kit Instruction

Catalogue No.

201-12-1978

Preface

Please carefully read this instruction before using. This ELISA kit is based on the principle of double-antibody sandwich technique to detect Human (ECSM1). Be used only for research purposes, not be used for medical diagnosis.

Full Name

Human Endothelial cell-specific Molecule 1(ECSM1) ELISA Kit

Intended Use

This kit is used to assay the Endothelial cell-specific Molecule 1(ECSM1) in the sample of human's serum, blood plasma, and other related tissue Liquid.

Test principle

The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Endothelial cell-specific Molecule 1(ECSM1) in samples. Add Endothelial cell-specific Molecule 1(ECSM1) to monoclonal antibody Enzyme well which is pre-coated with Human Endothelial cell-specific Molecule 1(ECSM1) monoclonal antibody, incubation; then, add



Endothelial cell-specific Molecule 1 (ECSM1) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance Endothelial cell-specific Molecule 1 (ECSM1) of sample were positively correlated.

Materials supplied in the Test Kit

1	Standard (2400ng/L)	0.5ml
2	Standard diluent	3m1
3	Microelisa Stripplate	12well×8strips
4	Str- HRP-Conjugate Reagent	6m1
5	30×wash solution	20m1
6	Biotin-ECSM1 Ab	1ml
7	Chromogen Solution A	6m1
8	Chromogen Solution B	6m1
9	Stop Solution	6m1
10	Instruction 1	
11	Closure plate membrane	2
12	Sealed bags	1

Materials required but not supplied

- 1. 37 ℃ incubator
- 2. Standard Enzyme reader



- 3. Precision pipettes and Disposable pipette tips
- 4. Distilled water
- 5. Disposable tubes for sample dilution
- 6. Absorbent paper

Important Notes

- 1. Beening taken out from the 2-8°C environment, the kit should be balanced 30 minutes in the ambient temperature then use. If the Coated plates of Enzyme haven't been used up after opened, the remaining plates should be
- stored in Sealed bag.
- 2. For each step, add Sample with sample injector which should be calibrated frequently, in order to avoid unnecessary experimental tolerance.
- 3. he operation shall be carried out accordance to the instructions strictly. And test results must be based on the readings of the Enzyme reader.
- 4. In order to avoid cross-contamination, it is forbidden to re-use the suction head and seal plate membrane in your hands.
- 5. All samples, washing buffer and each kind of reject should according to infective material process.
- 6. The idle agents shall be put up or covered. Do not use reagent with different batches. And use them before expired date.
- 7. The substrate B is light-sensitive. Prolonged exposure to light is forbidden.

Washing method

Manually washing method: shake away the remain liquid in the enzyme plates;



place some bibulous papers on the test-bed, and flap the plates on the upside down strongly. Inject at least 0.35ml after-dilution washing solution into the well, and marinate 1~2 minutes. Repeat this process according to your requirements.

<u>Automatic washing method:</u> if there is automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

Specimen requirements

- 1. Can't detect the sample which contain NaN3, because NaN3 inhibits HRP active
- 2. extract as soon as possible after Specimen collection, and according to the relevant literature, and should be experiment as soon as possible after the extraction. If it can't, specimen can be kept in −20 ℃ to preserve, Avoid repeated freeze-thaw cycles.
- 3. <u>serum-</u>coagulation at room temperature 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 4. <u>plasma</u>-use suited EDTA or citrate plasma as an anticoagulant, mix 10-20 mins, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.
- 5. <u>Urine-collect</u> sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again. The Operation of Hydrothorax and cerebrospinal fluid Reference to it.
- 6. <u>cell culture supernatant</u>-detect secretory components, collect sue a sterile container, centrifugation 20-min at the speed of 2000-3000 r.p.m.



remove supernatant, detect the composition of cells, Dilut cell suspension with PBS (PH7.2-7.4), Cell concentration reached 1 million / ml, repeated freeze-thaw cycles, damage cells and release of intracellular components, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, If precipitation appeared, Centrifugal again.

7. <u>Tissue samples</u>— After cutting samples, check the weight, add PBS (PH7.2-7.4), Rapidly frozen with liquid nitrogen, maintain samples at 2-8°C after melting, add PBS (PH7.4), Homogenized by hand or Grinders, centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

Assay procedure

1. Standard dilution:

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1200ng/	Standard No. 5	120µ 1 Original Standard + 120µ 1 Standard diluents
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600ng/L	Standard No. 4	120µ 1 Standard No.5 + 120µ 1 Standard diluents
300ng/L	Standard No.3	120µ 1 Standard No.4 + 120µ 1 Standard diluent
150ng/L	Standard No. 2	120µ 1 Standard No.3 + 120µ 1 Standard diluent
75ng/L	Standard No. 1	120µ 1 Standard No.2 + 120µ 1 Standard diluent

- 2. The quantity of the plates depends on the quantities of to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample shall be made according to your required quantity, and try to use the duplicated well as possible.
- 3. Inject samples:
 - ① Blank well: don't add samples and ECSM1-antibody labeled



with biotin, Streptavidin-HRP, only Chromogen solution A and B, and stop solution are allowed; other operations are the same.

- ② Standard wells: add standard 50μ 1, Streptavidin-HRP 50μ 1(since the standard already has combined biotin antibody, it is not necessary to add the antibody);
- ③ To be test wells: add sample 40μ 1, and then add both ECSM1 -antibody 10μ 1 and Streptavidin-HRP 50μ 1. Then seal the sealing memberance, and gently shaking, incubated 60 minutes at 37 °C.
- 4. Confection: dilute 30 times the 30×washing concentrate with distilled water as standby.
- 5. Washing: remove the memberance carefully, and drain the liquid, shake away the remaining water.
- 6. Add chromogen solution A 50μ 1, then chromogen solution B 50μ 1 to each well. Gently mixed, incubate for 10 min at 37° C away from light.
- 7. Stop: Add Stop Solution 50μ 1 into each well to stop the reaction(the blue changes into yellow immediately).
- 8. Final measurement: Take blank well as zero, measure the optical densit (OD) under 450 nm wavelength which should be carried out within 15min after adding the stop solution.
- 9. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use kinds of software to make calculations.



Summary procedures

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minutes at 37 ℃

1

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₽

Add stop solution

1

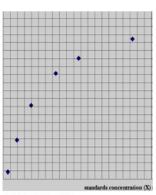
measure the OD value within 10min

1

Calculation

Calculate

Take the standard density as the horizontal, the OD value for the vertical, draw the standard curve on graph paper, Find out the corresponding density according to the sample OD value by the Sample curve (the result is the sample density)



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or calculate the straight line regression equation of the standard curve with the standard density and the OD value, with the sample OD value in the equation, calculate the sample density esult



Sensitivity, Assay range

Sensitivity: 7. 506ng/L(The sensitivity of this assay was defined as the lowest protein concentration that could be differentiated from zero. It was determined by sub-tracing two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.)

Assay range: $8ng/L \rightarrow 2000ng/L$

Specificity

This assay has high sensitivity and excellent specificity for detection of ECSM1. No significant cross-reactivity or interference between ECSM1 and analogues was observed.

- * Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between ECSM1 and all the analogues, therefore, cross reaction may still exist.
- Sample linear regression with the expected concentration of the correlation coefficient R is over 0.95

Intra-assay Precision: 3 samples with low, middle and high level human ECSM1 were tested 20 times on one plate, respectively.

Inter-assay Precision: 3 samples with low, middle and high level human ECSM1 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/meanX100 Intra-Assay: CV<10% Inter-Assay: CV<12%

Package size

96T perbox

validity&Storage

six months $(2-8^{\circ})$