



Human soluble VAP-1 ELISA Kit

User's Manual

**For Research Use Only, Not for use in diagnostic procedures**

ELISA Kit for Measuring Human soluble VAP-1

# CircuLex Human soluble VAP-1 ELISA Kit

Cat# CY-8104

Intended Use.....	1
Storage.....	1
Introduction.....	2
Principle of the Assay.....	2-3
Materials Provided.....	3
Materials Required but not Provided.....	4
Precautions and Recommendations.....	5
Sample Collection and Storage.....	6
Detailed Protocol.....	7-8
Calculations.....	9
Measurement Range.....	9
Troubleshooting.....	9
Reagent Stability.....	9
Assay Characteristics.....	10-12
Example of Test Results.....	13
References.....	14-15
License.....	15
Related Products.....	15

## Intended Use

The CycLex Research Product **CircuLex Human soluble VAP-1 ELISA Kit** is used for the quantitative measurement of human soluble VAP-1 in serum, plasma and other biological media.

**This assay kit is for research use only and not for use in diagnostic or therapeutic procedures.**

## Storage

- Upon receipt store all components at 4°C.
- Don't expose reagents to excessive light.

---

## **Introduction**

---

Vascular adhesion protein-1 (VAP-1), which is identical to amine oxidase, copper-containing 3 (AOC3), is a type II integral membrane protein with adhesion properties and amine oxidase activity (1-3). In vitro, VAP-1 catalyzes the oxidative deamination of small primary amines such as methylamine, benzylamine and aminoacetone in a reaction that produces an aldehyde, ammonia and H<sub>2</sub>O<sub>2</sub> (4-6). VAP-1 expression is highest in the endothelium of lung, heart, and intestine, but low in tissues such as brain, spleen, kidney, and liver (7-9). VAP1 vascular expression is regulated at sites of inflammation through its release from intracellular granules in which the protein is stored (10).

The concentration of soluble VAP-1 in serum is significantly higher in diabetes and obesity patients (11-13). In vitro, soluble VAP-1 enhances lymphocyte adhesion to endothelial cells, possibly participating in the enhanced lymphocyte adhesion capacity that is implicated in some inflammatory liver diseases (14) and the cardiovascular complications associated with diabetes or obesity (12, 15). Thus VAP-1 can function as a significant prognostic marker for certain cancers and cardiovascular diseases. Inhibition of VAP-1 can protect against inflammation related damage to certain injured tissues (13).

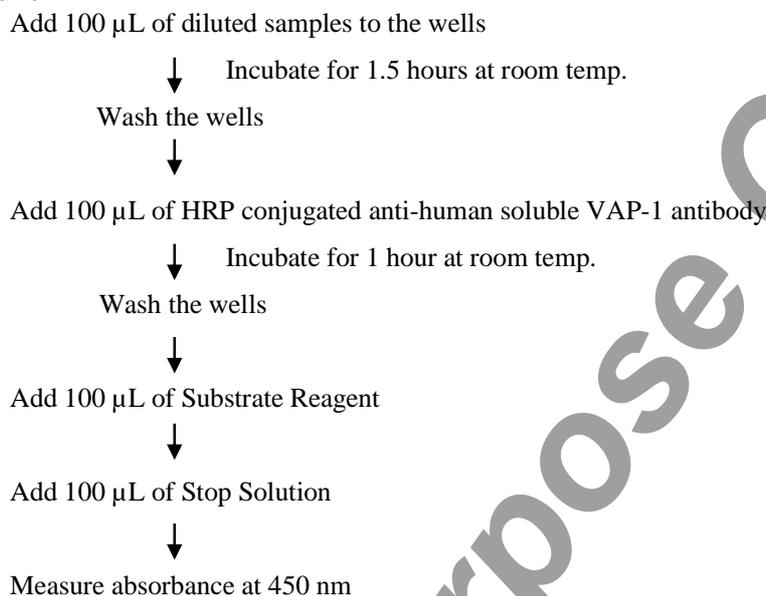
---

## **Principle of the Assay**

---

The CycLex Research Product **CircuLex Human soluble VAP-1 ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Human soluble VAP-1 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human soluble VAP-1 present. After washing away any unbound substances, an HRP conjugated antibody specific for human soluble VAP-1 is added to the wells. Following a wash to remove any unbound antibody HRP conjugate, the remaining conjugate is allowed to react with the substrate H<sub>2</sub>O<sub>2</sub>-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of human soluble VAP-1. A standard curve is constructed by plotting absorbance values versus human soluble VAP-1 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

### Summary of Procedure



---

### Materials Provided

---

All samples and standards should be assayed in duplicate. The following components are supplied and are sufficient for the one 96-well microplate kit.

**Microplate:** One microplate supplied ready to use, with 96 wells (12 strips of 8-wells) in a foil, zip-lock bag with a desiccant pack. Wells are coated with anti-human soluble VAP-1 monoclonal antibody as a capture antibody.

**10X Wash Buffer:** One bottle containing 100 mL of 10X buffer containing 1.3% Tween®-20

**Dilution Buffer:** One bottle containing 50 mL of 1X buffer; use for reconstitution of human soluble VAP-1 Standard and sample dilution. Ready to use.

**Human soluble VAP-1 Standard:** One vial containing 200 ng of lyophilized recombinant human soluble VAP-1.

**HRP conjugated Detection Antibody:** One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-human soluble VAP-1 monoclonal antibody. Ready to use.

**Substrate Reagent:** One bottle containing 20 mL of the chromogenic substrate, tetra-methylbenzidine (TMB). Ready to use.

**Stop Solution:** One bottle containing 20 mL of 1 N H<sub>2</sub>SO<sub>4</sub>. Ready to use.

---

## Materials Required but not Provided

---

- **Pipettors:** 2-20  $\mu$ L, 20-200  $\mu$ L and 200-1000  $\mu$ L precision pipettors with disposable tips
- **Precision repeating pipettor**
- **Orbital microplate shaker**
- **Microcentrifuge and tubes** for sample preparation
- **Vortex mixer**
- **Microplate washer:** optional (Manual washing is possible but not preferable)
- **Plate reader:** capable of measuring absorbance in 96-well plates at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. The plate can also be read at a single wavelength of 450 nm, which will give a somewhat higher reading.
- **Software package facilitating data generation and analysis: optional**
- **500 or 1000 mL graduated cylinder**
- **Reagent reservoirs**
- **Deionized water of the highest quality**
- **Disposable paper towels**

---

## Precautions and Recommendations

---

- Allow all the components to come to room temperature before use.
- All microplate strips that are not immediately required should be returned to the zip-lock pouch, which must be carefully resealed to avoid moisture absorption.
- Do not use kit components beyond the indicated kit expiration date.
- Use only the microtiter wells provided with the kit.
- Rinse all detergent residues from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents used in this kit contain  $\text{NaN}_3$  as preservatives. Care should be taken to avoid direct contact with these reagents.
- 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.
- Dispose of tetra-methylbenzidine (TMB) containing solutions in compliance with local regulations.
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide.
- Wear gloves and eye protection when handling immunodiagnostic materials and samples of human origin, and these reagents. In case of contact with the Stop Solution and the Substrate Solution, wash skin thoroughly with water and seek medical attention, when necessary.
- **Biological 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.**
- **CAUTION: Sulfuric Acid is a strong acid. Wear disposable gloves and eye protection when handling Stop Solution.**

---

## Sample Collection and Storage

---

**Serum:** Use a serum separator tube and allow samples to clot for  $60 \pm 30$  minutes. Centrifuge the samples at 4°C for 10 minutes at 1,000 x g. Remove serum and assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of serum may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

**Plasma:** Collect plasma using EDTA-Na<sub>2</sub> as the anticoagulant. If possible, collect the plasma into a mixture of EDTA-Na<sub>2</sub> and FUT-175 (Futhan) to stabilize the sample against spontaneous *in vitro* complement activation. Immediately centrifuge samples at 4°C for 15 minutes at 1,000 x g. Assay immediately or store samples on ice for up to 6 hours before assaying. Aliquots of plasma may also be stored at below -70°C for extended periods of time. Avoid repeated freeze-thaw cycles.

**Note:** Citrate plasma has not been validated for use in this assay.

**Cell culture supernatant:** Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

**Other biological samples:** Remove any particulates by centrifugation and assay immediately or aliquot and store samples at below -70°C. Avoid repeated freeze-thaw cycles.

**NOTE:** Although we suggest to conduct experiments as outlined above, the optimal experimental conditions will vary depending on the parameters being investigated, and must be determined by the individual user. **NO WARRANTY OR GUARANTEE OF PERFORMANCE USING THESE PROCEDURES IS MADE OR IMPLIED.**

## Detailed Protocol

The CycLex Research Product **CircuLex Human soluble VAP-1 ELISA Kit** is provided with removable strips of wells so the assay can be carried out on separate occasions using only the number of strips required for the particular determination. Since experimental conditions may vary, an aliquot of the Standard within the kit should be included in each assay as a calibrator. Disposable pipette tips and reagent troughs should be used for all liquid transfers to avoid cross-contamination of reagents or samples.

### Preparation of Working Solutions

All reagents need to be brought to room temperature prior to the assay. Assay reagents are supplied ready-to-use, with the exception of **10X Wash Buffer** and **Human soluble VAP-1 Standard**.

1. Prepare a working solution of Wash Buffer by adding 100 mL of the **10X Wash Buffer** to 900 mL of deionized (distilled) water. Mix well. Store at 4°C for two weeks or -20°C for long-term storage.
2. Reconstitute **Human soluble VAP-1 Standard** with **1 mL** of **Dilution Buffer**. The concentration of the human soluble VAP-1 in vial should be **200 ng/mL**, which is referred as a **Master Standard** of human soluble VAP-1.

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 20 ng/mL standard (Std.1) serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.1	100 µL of Master Standard (200 ng/mL)	900 µL	20.0 ng/mL
Std.2	300 µL of Std. 1 (20.0 ng/mL)	300 µL	10.0 ng/mL
Std.3	300 µL of Std. 2 (10.0 ng/mL)	300 µL	5.0 ng/mL
Std.4	300 µL of Std. 3 (5.0 ng/mL)	300 µL	2.5 ng/mL
Std.5	300 µL of Std. 4 (2.5 ng/mL)	300 µL	1.25 ng/mL
Std.6	300 µL of Std. 5 (1.25 ng/mL)	300 µL	0.625 ng/mL
Std.7	300 µL of Std. 6 (0.625 ng/mL)	300 µL	0.313 ng/mL
Blank	-	300 µL	0 ng/mL

**Note:** Do not use a repeating pipette. Change tips for every dilution. Unused portions of Master Standard should be aliquoted and stored at below -70°C immediately. Avoid multiple freeze and thaw cycles.

### Sample Preparation

- Serum and plasma samples require 50- to 100-fold dilution.
- Cell culture supernatant samples require neat to 10-fold dilution.

**Standard Assay Procedure for Human soluble VAP-1**

1. Remove the appropriate number of microtiter wells from the foil pouch and place them into the well holder. Return any unused wells to the foil pouch, refold, seal with tape and store at 4°C.
2. Dilute samples with Dilution Buffer. (See "Sample Preparation" above.)
3. Pipette **100 µL** of **Standard Solutions (Std1-Std7, Blank)** and **diluted samples** in duplicates, into the appropriate wells.
4. Incubate the plate at room temperature (ca.25°C) for 1.5 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
5. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
6. Add **100 µL** of **HRP conjugated Detection Antibody** into each well.
7. Incubate the plate at room temperature (ca.25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
8. Wash 4-times by filling each well with Wash Buffer (350 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or microplate washer.
9. Add **100 µL** of **Substrate Reagent**. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminum foil is recommended. Return Substrate Reagent to 4°C immediately after the necessary volume is removed
10. Incubate the plate at room temperature (ca.25°C) for 10-20 minutes, shaking at ca. 300 rpm on an orbital microplate shaker. The incubation time may be extended up to 30 minutes if the reaction temperature is below 20°C.
11. Add **100 µL** of **Stop Solution** to each well in the same order as the previously added Substrate Reagent.
12. Measure absorbance in each well using a spectrophotometric microplate reader at dual wavelengths of 450/540 nm. Dual wavelengths of 450/550 or 450/595 nm can also be used. Read the microplate at 450 nm if only a single wavelength can be used. Wells must be read within 30 minutes of adding the Stop Solution.

**Note-1:** Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

**Note-2:** Reliable standard curves are obtained when either O.D. values do not exceed 0.25 units for the blank (zero concentration), or 3.0 units for the highest standard concentration. The plate should be monitored at 5-minute intervals for approximately 30 minutes.

**Note-3:** If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine human soluble VAP-1 concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

---

## Calculations

---

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the human soluble VAP-1 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding human soluble VAP-1 concentration. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

1. The dose-response curve of this assay fits best to a sigmoidal 4-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4-parameter logistic function. It is important to make an appropriate mathematical adjustment to accommodate for the dilution factor.
2. Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus log of the known concentration (X) of calibrators, using the 4-parameter function. Alternatively, the logit log function can be used to linearize the calibration curve (i.e. logit of absorbance (Y) is plotted versus log of the known concentration (X) of calibrators).

---

## Measurement Range

---

The measurement range is 0.313 ng/mL to 20 ng/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer in higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the human soluble VAP-1 concentration.

---

## Troubleshooting

---

1. The human soluble VAP-1 Standard should be run in duplicate, using the protocol described in the **Detailed Protocol**. Incubation times or temperatures significantly different from those specified may give erroneous results.
2. Poor duplicates, accompanied by elevated values for wells containing no sample, indicate insufficient washing. If all instructions in the **Detailed Protocol** were followed accurately, such results indicate a need for washer maintenance.
3. Overall low signal may indicate that desiccation of the plate has occurred between the final wash and addition of Substrate Reagent. Do not allow the plate to dry out. Add Substrate Reagent immediately after wash.

---

## Reagent Stability

---

All of the reagents included in the CycLex Research Product **CircuLex human soluble VAP-1 ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the reconstituted human soluble VAP-1 Standard must be stored at below -70°C. Coated assay plates should be stored in the original foil bag sealed by the zip lock and containing a desiccant pack.

---

## Assay Characteristics

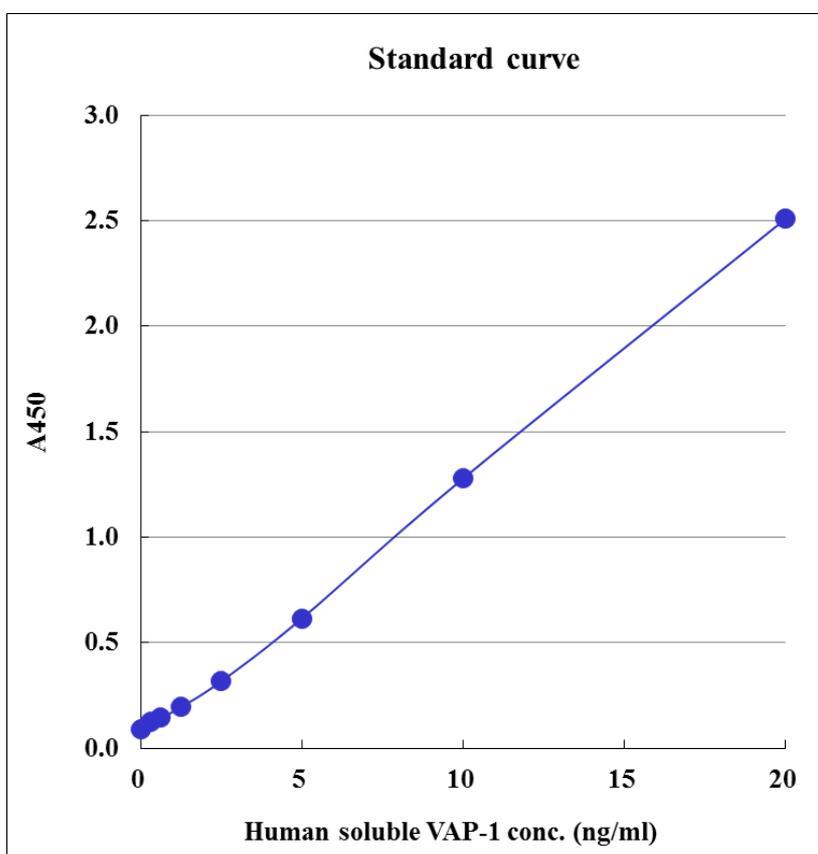
---

### 1. Sensitivity

The limit of detection (defined as such a concentration of human soluble VAP-1 giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank:  $A_{\text{blank}} + 3SD_{\text{blank}}$ ) is better than 0.145 ng/mL of sample.

\* Dilution Buffer is pipetted into blank wells.

### Typical Standard Curve



**2. Precision****Intra-assay Precision** (Precision within an assay)

Three samples\* of known concentration were tested eight times on one plate to assess intra-assay precision.

- Intra-assay (Within-Run, n=8) CV=3.9-6.5 %

\*Sample: Human serum

**Human soluble VAP-1 conc. (ng/mL)**

Sample No.	Sample 1	Sample 2	Sample 3
1	202.4	294.9	422.5
2	221.5	305.1	403.6
3	223.5	303.2	411.2
4	224.0	303.4	405.1
5	209.5	279.7	367.6
6	216.3	294.4	369.7
7	207.0	294.4	365.4
8	207.7	321.4	357.0
max.	224.0	321.4	422.5
min.	202.4	279.7	357.0
mean	214.0	299.6	387.8
SD	8.4	12.0	25.3
CV(%)	3.9	4.0	6.5

**Inter-assay Precision** (Precision between assays)

Three samples\* of known concentration were tested in four separate assays to assess inter-assay precision.

- Inter-assay (Run-to-Run, n=4) CV=4.4-6.1 %

\*Sample: Human serum

**Human soluble VAP-1 conc. (ng/mL)**

Sample No.	Sample 1	Sample 2	Sample 3
1	224.6	298.6	366.5
2	242.5	329.9	419.9
3	220.0	305.3	410.5
4	235.1	321.8	385.4
max.	242.5	329.9	419.9
min.	220.0	298.6	366.5
mean	230.6	313.9	395.6
SD	10.2	14.5	24.2
CV(%)	4.4	4.6	6.1

### 3. Spiking Recovery

Recombinant human soluble VAP-1 was added to samples\* at different concentrations.

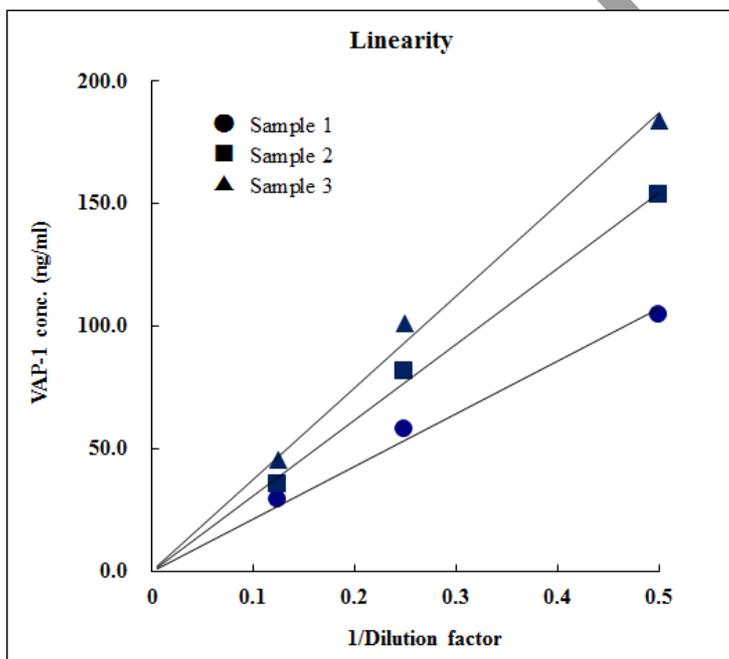
Sample	Spiked Concentration (ng/ml)	Observed Concentration (ng/ml)	Expected Concentration (ng/ml)	Recovery (%)
L	0	2.89	-	-
	1.25	3.74	4.14	90.6
	2.5	4.59	5.39	85.3
	5	6.16	7.89	78.2
M	0	3.24	-	-
	1.25	4.37	4.49	97.3
	2.5	5.29	5.74	92.2
	5	7.20	8.24	87.4
H	0	4.20	-	-
	1.25	4.87	5.45	89.2
	2.5	5.68	6.70	84.7
	5	7.08	9.20	76.9

\*Sample: Human serum

### 4. Linearity

Three samples\* were diluted with Dilution Buffer and assayed after dilution. The neat sample is set to 1. The results are summarized in the figure below.

\*Sample: Human serum



**Example of Test Results**

Fig.1 Human soluble VAP-1 concentrations in normal human sera (n=13)

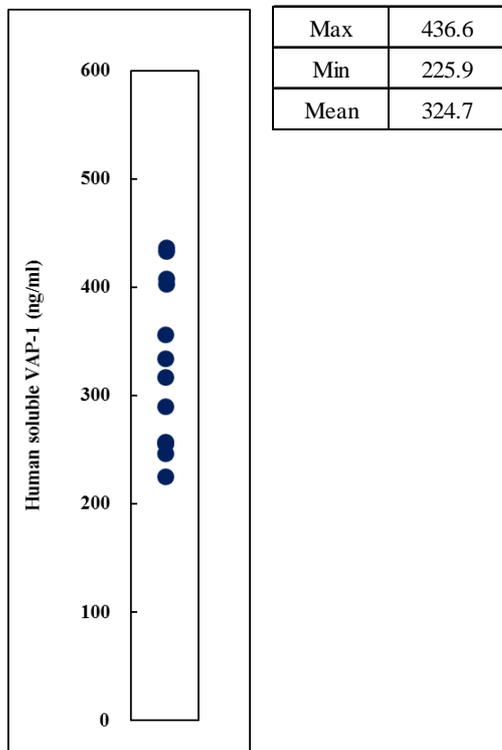
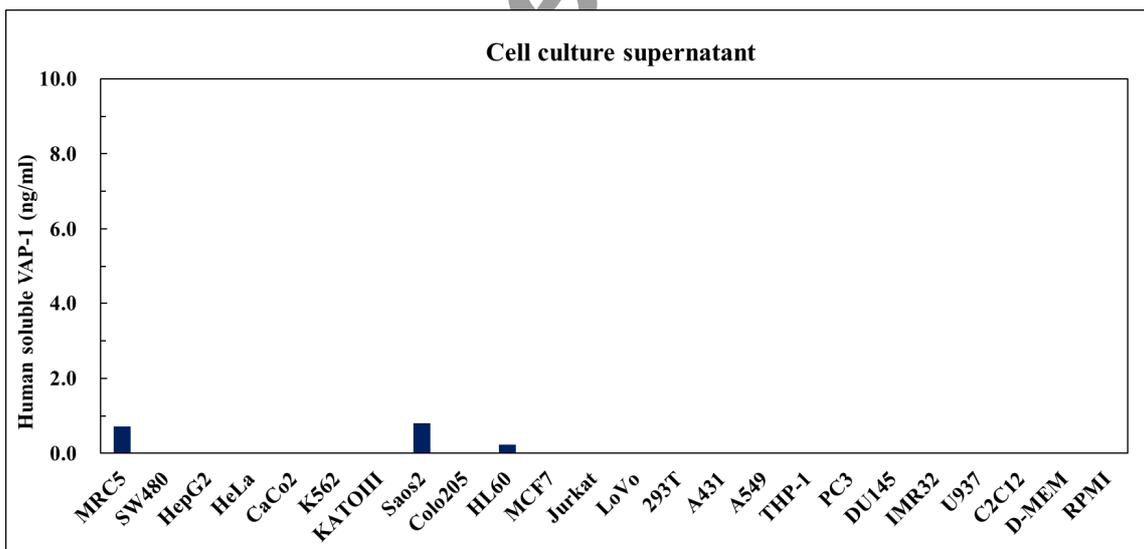


Fig.2 Human soluble VAP-1 concentrations in cell culture supernatants



---

## References

---

1. Springer, T. A. (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76, 301–314
2. Salmi, M., and Jalkanen, S. (1996) Human vascular adhesion protein-1 (VAP-1) is a unique sialoglycoprotein that mediates carbohydrate-dependent binding of lymphocytes to endothelial cells. *J. Exp. Med.* 183, 569–579
3. Smith, D. J., Salmi, M., Bono, P., Hellman, J., Leu, T., and Jalkanen, S. (1998) Cloning of vascular adhesion protein-1 reveals a novel multifunctional adhesion molecule. *J. Exp. Med.* 188, 17–27
4. Klinman, J. P., and Mu, D. (1994) Quinoenzymes in biology. *Annu. Rev. Biochem.* 63, 299–344
5. Lyles, G. A. (1996) Mammalian plasma and tissue-bound semicarbazide-sensitive amine oxidases: biochemical, pharmacological and toxicological aspects. *Int. J. Biochem. Cell Biol.* 28, 259–274
6. Wilmot, C. M., Hajdu, J., McPherson, M. J., Knowles, P. F., and Phillips, S. E. (1999) Visualization of dioxygen bound to copper during enzyme catalysis. *Science* 286, 1724–1728
7. Enrique-Taranco'n, G., Marti, L., Morin, N., Lizcano, J. M., Unzeta, M., Sevilla, L., Camps, M., Palacín, M., Testar, X., Carpe'ne', C., and Zorzano, A. (1998) Role of semicarbazide-sensitive amine oxidase on glucose transport and GLUT4 recruitment to the cell surface in adipose cells. *J Biol Chem.* 273, 8025-32.
8. Salmi, M., Yegutkin, G. G., Lehvonen, R., Koskinen, K., Salminen, T., and Jalkanen, S. (2001) A cell surface amine oxidase directly controls lymphocyte migration. *Immunity* 14, 265–276
9. Jaakkola, K., Kaunisma'ki, K., Tohka, S., Yegutkin, G., Va'tntinen, E., Havia, T., Pelliniemi, L. J., Virolainen, M., Jalkanen, S., and Salmi, M. (1999) Human vascular adhesion protein-1 in smooth muscle cells *Am. J. Pathol.* 155, 1953–1965
10. Arvilommi AM1, Salmi M, Jalkanen S. (1997) Organ-selective regulation of vascular adhesion protein-1 expression in man. *Eur J Immunol.* 27, 1794-800.
11. Abella A1, Garcı'a-Vicente S, Viguerie N, Ros-Baró A, Camps M, Palacín M, Zorzano A, Marti L. (2004) Adipocytes release a soluble form of VAP-1/SSAO by a metalloprotease-dependent process and in a regulated manner. *Diabetologia.* 47, 429-38.
12. Stolen, C. M., Madanat, R., Marti, L., Kari, S., Yegutkin, G. G., Sariola, H., Zorzano, A., and Jalkanen, S. (2004) Semicarbazide sensitive amine oxidase overexpression has dual consequences: insulin mimicry and diabetes-like complications. *FASEB J.* 18, 702–704
13. Kiss, J., Jalkanen, S., Fu'lo'p, F., Savunen, T., and Salmi, M. (2008) Ischemia-reperfusion injury is attenuated in VAP-1-deficient mice and by VAP-1 inhibitors. *Eur. J. Immunol.* 38, 3041–3049
14. Kurkijärvi R1, Adams DH, Leino R, Möttönen T, Jalkanen S, Salmi M.(1998) Circulating form of human vascular adhesion protein-1 (VAP-1): increased serum levels in inflammatory liver diseases. *J Immunol.* 161, 1549-57.

15. Gokturk, C., Sugimoto, H., Blomgren, B., Roomans, G. M., Forsberg-Nilsson, K., Oreland, L., and Sjoquist, M. (2007) Macrovascular changes in mice overexpressing human semicarbazide-sensitive amine oxidase in smooth muscle cells. *Am. J. Hypertens.* 20, 743–750

---

### **License**

---

This kit was developed and produced by CycLex Co., Ltd., Nagano, Japan, under license from R-Tech Ueno, Ltd., Japan.

---

### **Related Products**

---

\* **CircuLex Human LOXL2 ELISA Kit: Cat# CY-8100**

#### **PRODUCED BY**

**CycLex Co., Ltd.**  
**1063-103 Terasawaoka**  
**Ina, Nagano 396-0002**  
**Japan**  
**Fax: +81-265-76-7618**  
**e-mail: [info@cyclex.co.jp](mailto:info@cyclex.co.jp)**  
**URL: <http://www.cyclex.co.jp>**

CycLex/CircuLex products are supplied for research use only. CycLex/CircuLex products and components thereof may not be resold, modified for resale, or used to manufacture commercial products without prior written approval from CycLex Co., Ltd.. To inquire about licensing for such commercial use, please contact us via email.