



Human AIM/CD5L/Spα ELISA Kit

User's Manual

For Research Use Only, Not for use in diagnostic procedures

ELISA Kit for Measuring Free Form Human AIM/CD5L/Spα

# CircuLex Human AIM/CD5L/Spα ELISA Kit

Cat# CY-8080

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### Intended Use

The CycLex Research Product **CircuLex Human AIM/CD5L/Spα ELISA Kit** is used for the quantitative measurement of free form human AIM/CD5L/Spα 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.

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## Introduction

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AIM, also called CD5L/Spα, is a soluble 38 or 40-kDa glycoprotein expressed by macrophages present in lymphoid tissues (spleen, lymph node, thymus, and bone marrow), for which little functional and structural information is available (1). It belongs to the group B of the scavenger receptor cysteine-rich (SRCR) superfamily that includes the lymphocyte surface receptors CD5 and CD6 among others. AIM is able to bind to not only myelomonocytic and lymphoid cells (2), but also the surface of several Gram-positive and Gram-negative bacterial strains (3), which suggests that it may play an important role in the regulation of the innate and adaptive immune systems. It has been reported that AIM is present in IgM fraction, but not IgG or IgA fractions, from human serum (4). AIM is a circulating protein that may play a role in the homeostasis of IgM antibodies. In addition, it has been reported that AIM expression is induced in mouse macrophages in response to loading with highly oxidized low density lipoprotein (5), and that AIM is expressed in foam cells within atherosclerotic lesions. AIM may be feasible as a potential biomarker in the diagnosis and treatment of atopic dermatitis (6), although AIM was reported as candidate marker in asthma (7) and liver cirrhosis (8, 9)

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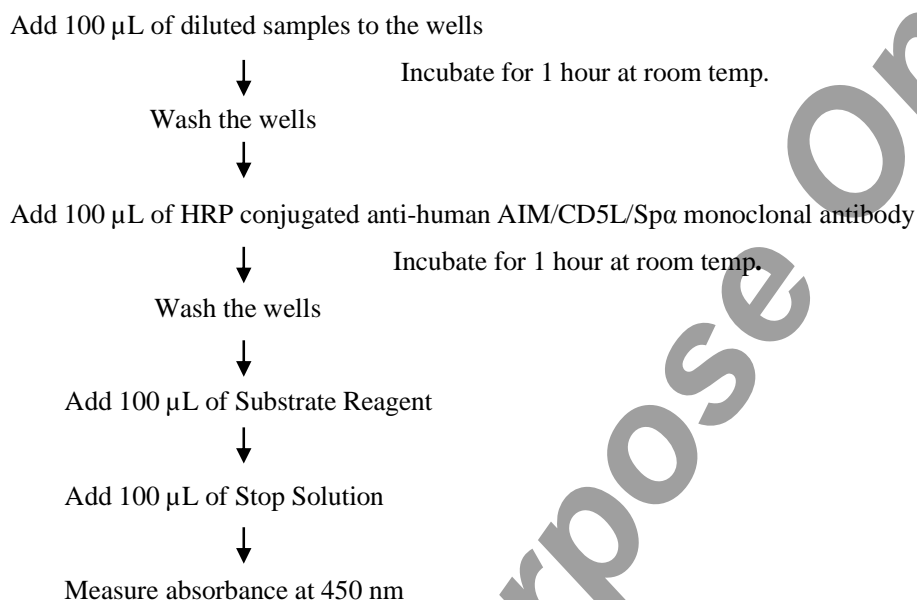
## Principle of the Assay

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The CycLex Research Product **CircuLex Human AIM/CD5L/Spα ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human AIM/CD5L/Spα is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any human AIM/CD5L/Spα present. After washing away any unbound substances, an HRP conjugated monoclonal antibody specific for human AIM/CD5L/Spα 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 AIM/CD5L/Spα. A standard curve is constructed by plotting absorbance values versus human AIM/CD5L/Spα concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

The CycLex Research Product **CircuLex Human AIM/CD5L/Spα ELISA Kit** is designed to measure the concentration of free form human AIM/CD5L/Spα in human serum/plasma or conditioned medium. Most of human AIM/CD5L/Spα associates with IgM and other serum proteins in the circulation. **CircuLex Human AIM/CD5L/Spα ELISA Kit** can measure only free form human AIM/CD5L/Spα concentration using standard assay procedure. The concentration of free form human AIM/CD5L/Spα showed a clear correlation with one of total human AIM/CD5L/Spα (See Fig.2, page 15 and 16). However treatment of sample with reducing reagent such as dithiothreitol and urea allow measuring relative concentration of total human AIM/CD5L/Spα.

### Summary of Procedure



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### Materials Provided

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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 AIM/CD5L/Spα antibody as a capture antibody.

**10X Wash Buffer:** One bottle containing 100 mL of 10X buffer containing 2% Tween®-20

**Dilution Buffer:** One bottle containing 50 mL of 1X buffer; use for reconstitution of Human AIM/CD5L/Spα Standard and sample dilution. Ready to use.

**Human AIM/CD5L/Spα Standard:** One vial containing 400 ng of lyophilized recombinant human AIM/CD5L/Spα

**20x HRP conjugated Detection Antibody:** One vial containing 600 µL of HRP (horseradish peroxidase) conjugated anti-human AIM/CD5L/Spα monoclonal antibody (KS-3G7).

**Conjugate Dilution Buffer:** One bottle containing 12 mL of Conjugate Dilution Buffer.

**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.

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### **Materials Required but not Provided**

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- **Reducing agent (10 mM DTT and 4 M Urea):** optional (for measurement of total human AIM/CD5L/Sp $\alpha$ )
- **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**

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## Precautions and Recommendations

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- 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 residue from glassware.
- Use deionized water of the highest quality.
- Do not mix reagents from different kits.
- The buffers and reagents in this kit may contain preservatives or other chemicals. 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.**

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## Sample Collection and Storage

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**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 Futhan5 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.

**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.

## Detailed Protocol

The CycLex Research Product **CircuLex Human AIM/CD5L/Spα 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 human AIM/CD5L/Spα 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 AIM/CD5L/Spα 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 (ddH<sub>2</sub>O). Mix well. Store at 4°C for two weeks or -20°C for long-term storage.
2. Prepare **HRP conjugated Detection Antibody** by 20-fold diluting **20X HRP conjugated Detection Antibody** with Conjugate Dilution Buffer at the time of assay.

*Prepare appropriate volume for your assay. Discard any unused HRP-conjugated Detection Antibody after diluted.*

3. Reconstitute **Human AIM/CD5L/Spα Standard** with 1.0 mL of ddH<sub>2</sub>O. The concentration of the human AIM/CD5L/Spα in vial should be 400 ng/mL, which is referred as a **Master Standard** of human AIM/CD5L/Spα.

Prepare Standard Solutions as follows:

Use the **Master Standard** to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 100 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	150 µL of Master Standard	450 µL	100 ng/mL
Std.2	300 µL of Std. 1 (100 ng/ml)	300 µL	50 ng/mL
Std.3	300 µL of Std. 2 (50 ng/ml)	300 µL	25 ng/mL
Std.4	300 µL of Std. 3 (25 ng/ml)	300 µL	12.5 ng/mL
Std.5	300 µL of Std. 4 (12.5 ng/ml)	300 µL	6.25 ng/mL
Std.6	300 µL of Std. 5 (6.25 ng/ml)	300 µL	3.13 ng/mL
Std.7	300 µL of Std. 6 (3.13 ng/ml)	300 µL	1.57 ng/mL
Blank	-	300 µL	0 ng/mL

**Note:** Do not use a Repeating pipette. Change tips for every dilution. Wet tip with Dilution Buffer before dispensing. 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 a 50-fold dilution.  
e.g. 6 µL of sample + 294 µL of Dilution Buffer
- Other biological samples require neat to appropriate dilution.

### Standard Assay Procedure for Free Form Human AIM/CD5L/Spα

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 hour, 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 than 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.



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**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 AIM/CD5L/Spα concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

**Assay Procedure for Total Human AIM/CD5L/Spα**

1. Add **10 μL** of **serum sample** to **90 μL** of **Reducing agent (10 mM DTT and 4 M Urea)**. Mix well.
2. Stand at room temperature for 30 min. (Resultant sample is referred to DTT-treated serum sample)
3. Dilute DTT-treated serum sample **1:400** with **Dilution Buffer** (e.g. First: Make 20-fold dilution (10 μL of sample + 190 μL of Dilution Buffer). Second: Make 400-fold dilution (15 μL of 20-fold diluted sample + 285 μL Dilution Buffer). Final dilution factor should be 4,000.
4. Go to step 3 in "Standard Assay Procedure for Free Form Human AIM/CD5L/Spα". Follow later steps.

**Note:** It should be noted that the total human AIM/CD5L/Spα concentration calculated from this procedure is just only relative value. There is no good linearity.

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**Calculations**

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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 AIM/CD5L/Spα 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 AIM/CD5L/Spα 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 four-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).

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## Measurement Range

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The measurement range is 1.57 ng/mL to 100 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 human AIM/CD5L/Spα concentration.

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## Troubleshooting

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1. The AIM/CD5L/Spα 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.

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## Reagent Stability

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All of the reagents included in the CycLex Research Product **CircuLex Human AIM/CD5L/Spα 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 AIM/CD5L/Spα 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

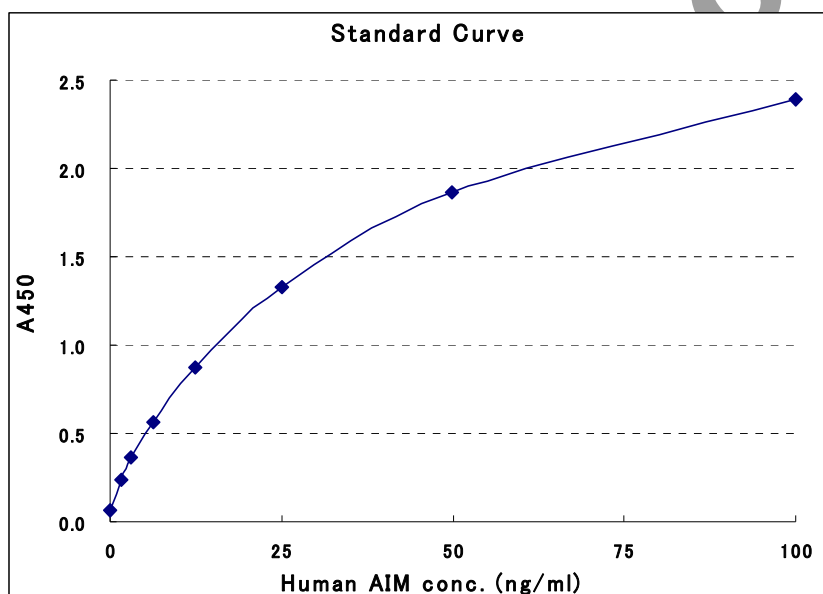
### 1. Sensitivity

The limit of detection (defined as such a concentration of human AIM/CD5L/Spα giving absorbance higher than mean absorbance of blank\* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 0.745 ng/ml of sample.

\* Dilution Buffer is pipetted into blank wells.

Eighty assays were evaluated and the minimum detectable dose (MDD) of human AIM/CD5L/Spα ranged from 0.916- 0.584 ng/mL. The mean MDD was 0.754 ng/mL. The MDD was determined by adding three standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

### Typical Standard Curve



## 2. Precision

### Intra-assay Precision (Precision within an assay)

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

- Intra-assay (Within-Run, n=14) CV=3.6-6.5 %

### Free form human AIM/CD5L/Spα conc. (ng/mL)

Serum No.	Serum 1	Serum 2	Serum 3
1	174.5	381.3	982.8
2	174.5	401.9	982.8
3	173.1	375.8	954.2
4	180.2	401.9	957.1
5	178.8	411.4	985.6
6	167.6	413.3	926.2
7	177.4	426.8	887.6
8	187.3	411.4	882.2
9	168.9	366.6	828.8
10	167.6	383.2	887.6
11	180.2	396.2	857.9
12	178.8	401.9	890.3
13	183	411.4	800.1
14	185.9	422.9	887.6
<b>MAX.</b>	187.3	426.8	985.6
<b>MIN.</b>	167.6	366.6	800.1
<b>MEAN</b>	<b>177.0</b>	<b>400.4</b>	<b>907.9</b>
<b>S.D.</b>	<b>6.3</b>	<b>17.9</b>	<b>58.7</b>
<b>C.V.</b>	<b>3.6%</b>	<b>4.5%</b>	<b>6.5%</b>

Inter-assay Precision (Precision between assays)

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

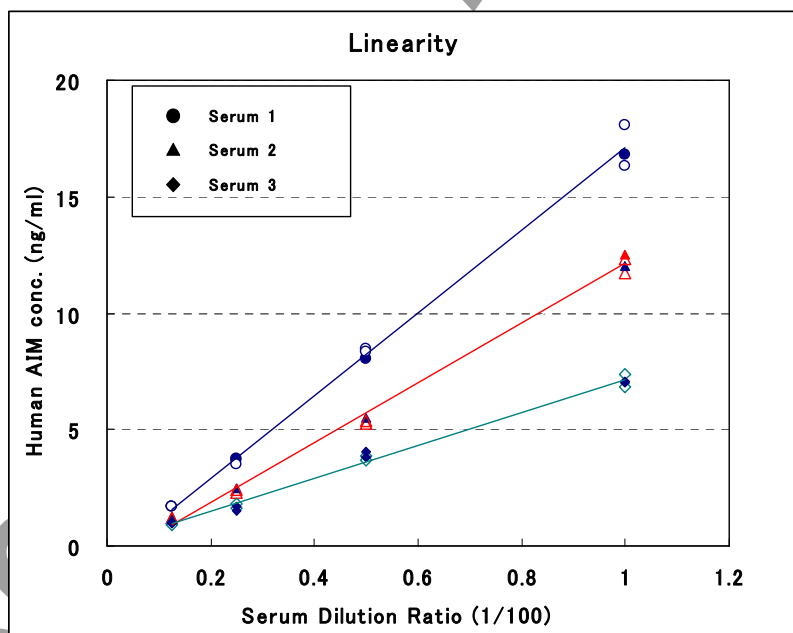
- Inter-assay (Run-to-Run, n=5) CV=2.1 - 5.7 %

**Free form human AIM/CD5L/Spα conc. (ng/mL)**

	Serum 1	Serum 2	Serum 3
1	172.1	389.4	931.3
2	171.5	396.8	818.6
3	168.4	422.1	912.0
4	177.6	399.7	941.9
5	169.2	385.6	939.2
MAX.	177.6	422.1	941.9
MIN.	168.4	385.6	818.6
<b>MEAN</b>	<b>171.8</b>	<b>398.7</b>	<b>908.6</b>
<b>S.D.</b>	<b>3.6</b>	<b>14.2</b>	<b>51.7</b>
<b>C.V.</b>	<b>2.1%</b>	<b>3.6%</b>	<b>5.7%</b>

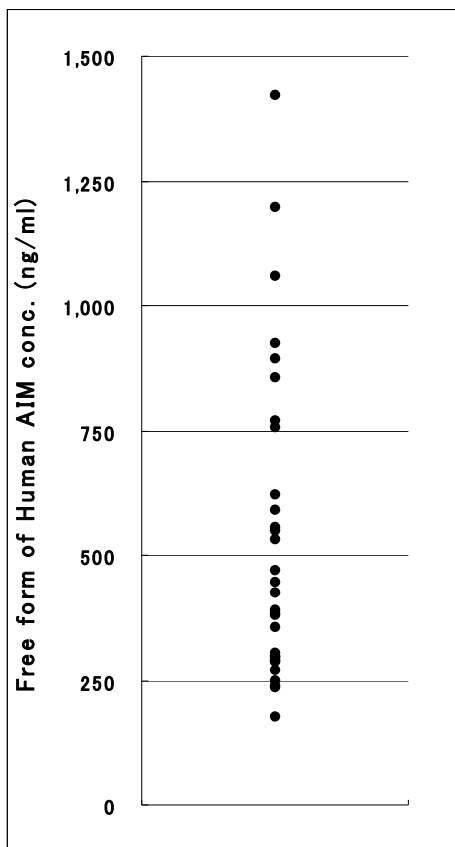
**3. Linearity**

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of free form human AIM/CD5L/Spα were serially diluted with the Dilution Buffer to produce samples with values within the dynamic range of the assay.



**Example of Test Results**

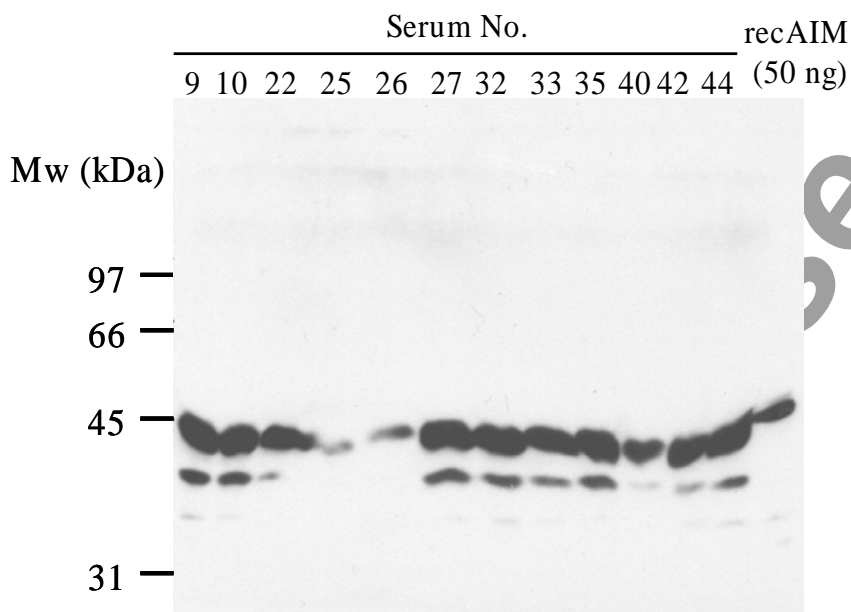
Fig.1 Concentrations of free form human AIM/CD5L/Spα in healthy Japanese volunteers' sera, n = 32 (Male: 18, Female: 14).



Normal Human Serum	
n	32
Average	531.7
Maximum	1421.7
Minnimum	175.2

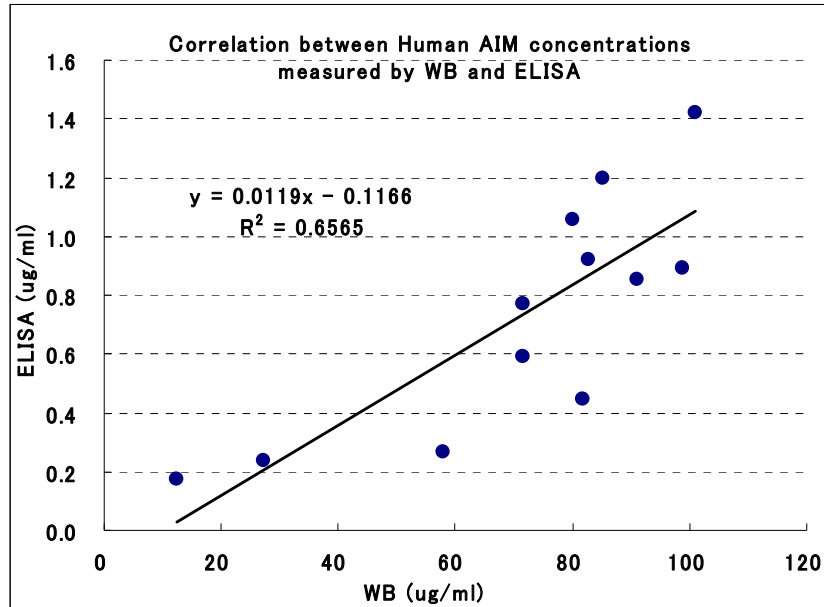
Fig.2 Correlation between concentrations of total AIM/CD5L/SPα measured by western blotting and those of free form AIM/CD5L/SPα measured by AIM/CD5L/Spα ELISA Kit.

I. Western blotting with anti-human AIM/CD5L/Spα polyclonal antibody.



II. Concentrations of total AIM/CD5L/Spα (measured by western blotting) and those of free form AIM/CD5L/Spα measured by CircuLex Human AIM/CD5L/Spα ELISA Kit.

Serum No.	AIM conc (ug/ml)	
	Measured by WB	Measured by ELISA
9	82.66	0.92
10	71.48	0.77
22	80.08	1.06
25	12.43	0.18
26	27.15	0.24
27	101.11	1.42
32	98.82	0.89
33	81.83	0.45
35	85.12	1.20
40	57.91	0.27
42	71.49	0.59
43	91.08	0.85





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## Related Products

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- \* CircuLex Mouse AIM/CD5L/Spα ELISA Kit: Cat# CY-8075
- \* CircuLex Human AIM/CD5L/Spα ELISA Kit: Cat# CY-8080
- \* Anti-Human AIM/CD5L/Spα polyclonal antibody: Cat# CY-P1036
- \* Human AIM/CD5L/Spα: Cat# CY-R2270

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