ELISA Kit for Measuring VILIP-1/2

CircuLex VILIP-1/2 ELISA Kit

Cat# CY-8106

Intended Use

The CycLex Research Product **CircuLex VILIP-1/2 ELISA Kit** is used for the quantitative measurement of VILIP-1/2 in tissue extract, cell lysate and other biological media, which are derived from rodent to human.

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

Visinin-like protein-1 (VILIP-1) is a neuronal calcium-binding protein (1) which has been re-identified as a marker of neuronal injury in large-scale gene-array analyses and in brain injury models (2). VILIP-1 contains four EF-hands and a double-stranded RNA binding domain involved in calcium-dependent signal transduction mechanisms in neurons (3). VILIP-1 is a cytoplasmic protein found almost exclusively in neurons of the central nervous system and human VILIP-1 shares 100% amino acid identity with the mouse and rat homologs. In addition, this evolutionarily well-conserved protein is widely expressed throughout the brain (4-6) but rarely detectable in other peripheral tissues (2, 7) except for pancreatic beta-cells (8). Increased VILIP-1 in beta-cells enhanced insulin secretion in a cAMP-associated manner, where down-regulation of VILIP-1 was accompanied by decreased cAMP accumulation but increased insulin content (8).

The concentration of VILIP-1 was increased in plasma of stroke patients and in cerebrospinal fluid in a rat model for stroke, suggesting that VILIP-1 is a potential and very promising biomarker for CNS damage (2, 9). VILIP-1 and YKL-40 (also called CHI3L1 (Chitinase-3-like protein 1)) in cerebrospinal fluid were reported predict biomarkers of Alzheimer's disease (AD) in human clinical cohort study (10).

Principle of the Assay

The CycLex Research Product **CircuLex VILIP-1/2 ELISA Kit** employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for VILIP-1/2 is pre-coated onto a microplate. Standards and samples are pipetted into the wells and the immobilized antibody binds any VILIP-1/2 present. After washing away any unbound substances, an HRP conjugated antibody specific for VILIP-1/2 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_2O_2- 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 VILIP-1/2. A standard curve is constructed by plotting absorbance values versus VILIP-1 concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

Summary of Procedure

Add 100 µL of diluted samples to the wells
↓ Incubate for 1 hour at room temp.
Wash the wells
↓
Add 100 µL of HRP conjugated anti-VILIP-1/2 antibody
↓ Incubate for 1 hour at room temp.
Wash the wells
↓
Add 100 µL of Substrate Reagent
↓
Add 100 µL of Stop Solution
↓
Measure absorbance at 450 nm

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-VILIP-1/2 monoclonal antibody as a capture antibody.

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

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

VILIP-1 Standard: One vial containing 200 ng of lyophilized recombinant VILIP-1.

HRP conjugated Detection Antibody: One bottle containing 12 mL of HRP (horseradish peroxidase) conjugated anti-VILIP-1/2 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_2SO_4. Ready to use.

Materials Required but not Provided

- **Pipettors:** 2-20 µL, 20-200 µL and 200-1000 µL 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

- **This kit can NOT distinguish between VILIP-1 and VILIP-2. See the "4.Cross-reactivity of CircuLex VILIP-1/2 ELISA Kit" in the section "Assay Characteristics" below**.
- 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 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

Cell lysates: Prepare cell lysates (see below). Assay immediately or store the samples on ice for a few hours before assaying. Aliquots of the samples may also be stored at below -70°C for extended periods of time. 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.

For reference

Serum: Use a serum separator tube and allow samples to clot for 60 ± 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_2 as the anticoagulant. If possible, collect the plasma into a mixture of EDTA-Na_2 and Futhan (FUT175) 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.

Preparation of Cell Lysate: Several extraction methods can be used for measurement cellular total VILIP-1/2. The following protocol has been shown to work with a number of different cell lines and is provided as an example of suitable methods. It is strongly advised that the user always perform an initial experiment to determine the proper dilution to be used in subsequent experiments. This need not be any more than a single time point assay using serial dilutions of the cell lysate. One eight well strip of the substrate plate should be sufficient for this initial experiment. All steps of cell lysate preparation should be performed at 4°C and recovered cell lysate should be kept at -70°C.

A. Cell Lysis Buffer
25 mM Tris HCl, pH 7.5, 250 mM NaCl, 10 % glycerol, 0.1 % NP-40, 5 mM EDTA-2Na (pH 7.5), 0.2 mM PMSF, 1 µg/mL pepstatin, 0.5 µg/mL leupeptin, 0.2 mM DTT

B. Preparation of poly-L-lysine coated plate
Coat the dish with 25 µg/mL poly-l-lysine (PLL) in PBS for 4-12 hours at 37°C. Subsequently go to a washing step with PBS.

C. Treatment of Cells

1. Plate adherent cells in PLL-coated Ø 3 cm dish at around 1 x 10^5 cells/dish.

2. Incubate the culture dish at 37°C overnight in CO_2 incubator.

3. Add appropriate amount of test compound and vehicle to each well. Incubate the culture dish at 37°C for appropriate time (drug treatment).

D. Cell Extraction

Note: This protocol has been successfully applied to A549 cell line. Users should optimize the cell extraction procedure for their own applications.

4. Wash cells three times with ice-cold PBS. Remove any remaining PBS by decanting. Invert the dish and blot it against clean paper towels. At this point the cells in the plate can be frozen at below -70°C and lysed at a later date.

5. Lyse the cells by adding 0.2 mL* of Cell Lysis Buffer for 60-90 minutes at 4°C, with rotating at ca. 300 rpm by an orbital microplate shaker.

   * *To get a rough idea you could adjust the cell concentration to around 1-2 x 10^6 cells/mL in Cell Lysis Buffer. Resulting protein concentration of the A549 cell lysate should be 1.2-2.0 mg/mL using this procedure.*

   * *The appropriate volume of Cell Lysis Buffer depends on the cell line, the cell number and the amount of total VILIP-1/2.*

6. Transfer the cell lysates to microcentrifuge tubes and centrifuge at 15,000 rpm for 5 minutes at 4°C.

7. Transfer the clear cell lysates to new microcentrifuge tubes. Dilute these cell lysates 4-16 times with Dilution Buffer. 100 µL of these diluted cell lysates are ready for assay. Go to the section "Standard Assay Procedure for VILIP-1/2" below.

Typical data using this protocol are shown in Fig.2 of the section "Example of Test Results" below.

The cell lysates can be stored at below -70°C. Avoid multiple freeze/thaw cycles. After thaw the cell lysates, Centrifuge at 15,000 rpm for 5 minutes at 4°C again since the cell lysates should be clear of any sediments or particulate matter.

NOTE: THE ABOVE PROCEDURES ARE INTENDED ONLY AS A GUIDELINE. 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 VILIP-1/2 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 VILIP-1 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 **VILIP-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 **VILIP-1 Standard** with **1 mL** of **Dilution Buffer**. The concentration of the VILIP-1 in vial should be **200 ng/mL**, which is referred as a **Master Standard** of VILIP-1.

Prepare Standard Solutions as follows:

Use the **Master Standard (200 ng/mL)** to produce **Std.0 (4 ng/mL)** and make a dilution series (below). Mix each tube thoroughly before the next transfer. The **Std.1 (400 pg/mL)** serves as the highest standard. The **Dilution Buffer** serves as the zero standard (Blank).

	Volume of Standard	Dilution Buffer	Concentration
Std.0	20 µL of **Master Standard (200 ng/mL)**	980 µL	**4 ng/mL**
Std.1	100 µL of Std.0 (4 ng/mL)	900 µL	400 pg/mL
Std.2	300 µL of Std.1 (400 pg/mL)	300 µL	200 pg/mL
Std.3	300 µL of Std.2 (200 pg/mL)	300 µL	100 pg/mL
Std.4	300 µL of Std.3 (100 pg/mL)	300 µL	50 pg/mL
Std.5	300 µL of Std.4 (50 pg /mL)	300 µL	25 pg/mL
Std.6	300 µL of Std.5 (25 pg/mL)	300 µL	12.5 pg/mL
Std.7	300 µL of Std.6 (12.5 pg /mL)	300 µL	6.25 pg /mL
Blank	-	300 µL	0 pg/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

Dilute samples with **Dilution Buffer**. Samples, e.g. tissue extracts and cell lysates, require the proper dilution ratio. Dilution ratio varies depending on the sample types and the isolation and extraction conditions.

Note: Central nervous systems contain VILIP-1/2 in a high concentration, several hundred ng/mg protein, and VILIP-1/2 is drastically induced in cell lines under certain conditions. Refer to the section "Example of Test Results" below.

Standard Assay Procedure for VILIP-1/2

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 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 VILIP-1/2 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 VILIP-1/2 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 VILIP-1/2 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 6.25 pg/mL to 400 pg/mL. Any sample reading higher than the highest standard should be diluted with Dilution Buffer to higher dilution and re-assayed. Dilution factors need to be taken into consideration in calculating the VILIP-1/2 concentration.

Troubleshooting

1. All samples and standards should be assayed 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

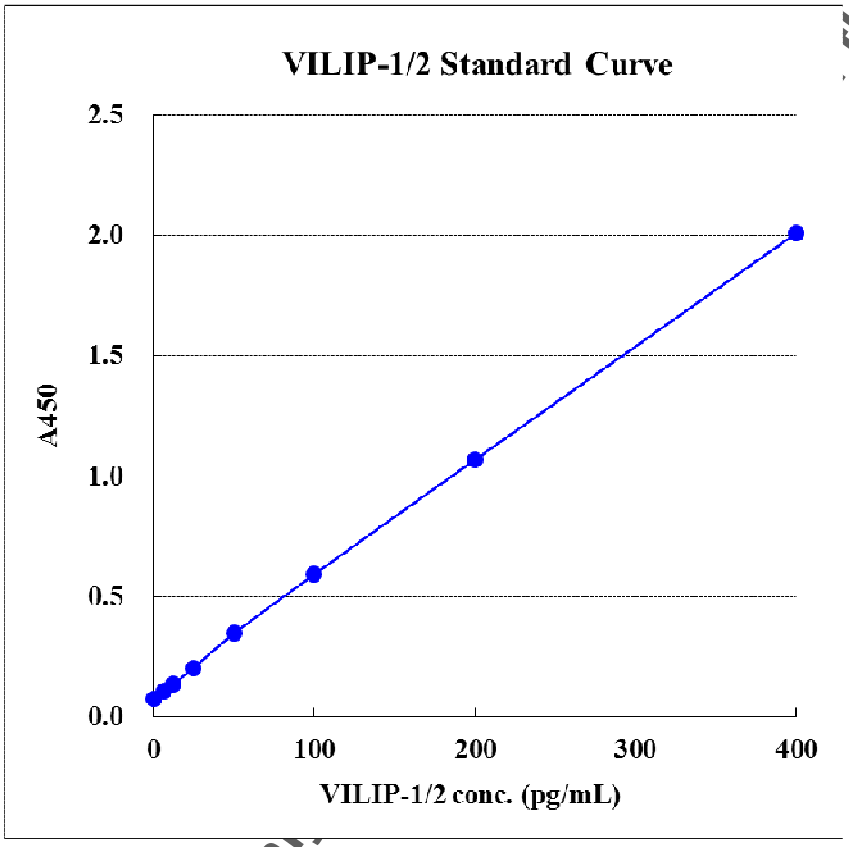
All of the reagents included in the CycLex Research Product **CircuLex VILIP-1/2 ELISA Kit** have been tested for stability. Reagents should not be used beyond the stated expiration date. Upon receipt, kit reagents should be stored at 4°C, except the reconstituted VILIP-1 Standard must be stored at below -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 VILIP-1 giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A blank + 3SD blank) is better than 5.7 pg/mL of sample.

* Dilution Buffer is pipetted into blank wells.

Typical Standard Curve



2. Precision

Intra-assay Precision (Precision within an assay)

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

• Intra-assay (Within-Run, n=8) CV=2.7-3.6 %

*Sample: rat and mouse brain extract

VILIP-1/2 concentration (pg/mL)

	Sample 1	Sample 2	Sample 3	Sample 4
1	63.4	71.6	141.7	303.9
2	59.3	68.2	132.4	294.1
3	62.4	70.3	131.4	302.8
4	62.2	72.1	134.9	316.4
5	66.9	71.8	137.2	317.9
6	64.8	69.0	131.0	303.2
7	63.8	69.2	134.1	299.8
8	65.1	75.1	137.3	309.2
MAX.	66.9	75.1	141.7	317.9
MIN.	59.3	68.2	131.0	294.1
MEAN	63.5	70.9	135.0	305.9
S.D.	2.3	2.2	3.6	8.1
C.V.	**3.6%**	**3.1%**	**2.7%**	**2.7%**

Inter-assay Precision (Precision between assays)

Four samples* of known concentration were tested in three separate assays to assess inter-assay precision.

• Inter-assay (Run-to-Run, n=3) CV=1.1-8.9 %

*Sample: rat and mouse brain extract

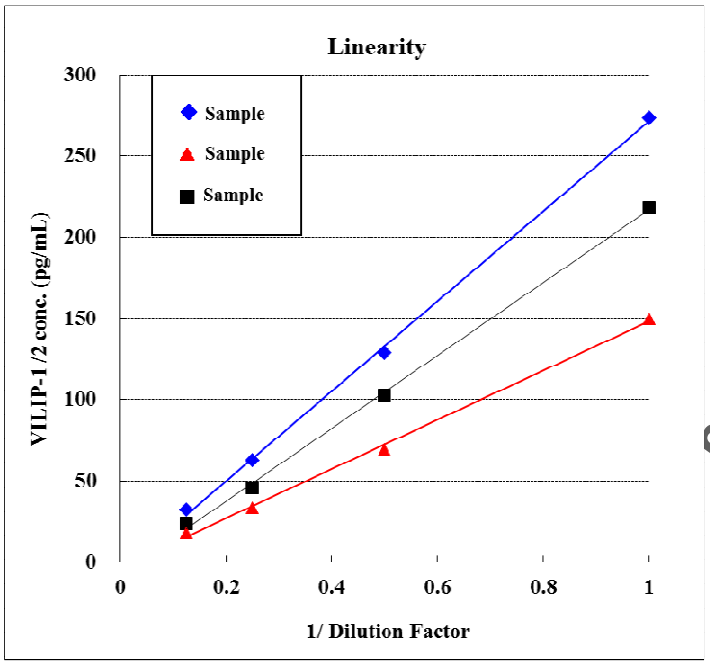
VILIP-1/2 concentration (pg/mL)

	Sample 1	Sample 2	Sample 3	Sample 4
Assay-1	63.5	70.9	135.0	305.9
Assay-2	62.0	69.4	128.8	287.7
Assay-3	62.1	70.3	126.1	256.0
MAX.	63.5	70.9	135.0	305.9
MIN.	62.0	69.4	126.1	256.0
MEAN	62.5	70.2	130.0	283.2
S.D.	0.84	0.75	4.55	25.24
C.V.	**1.3%**	**1.1%**	**3.5%**	**8.9%**

3. Linearity

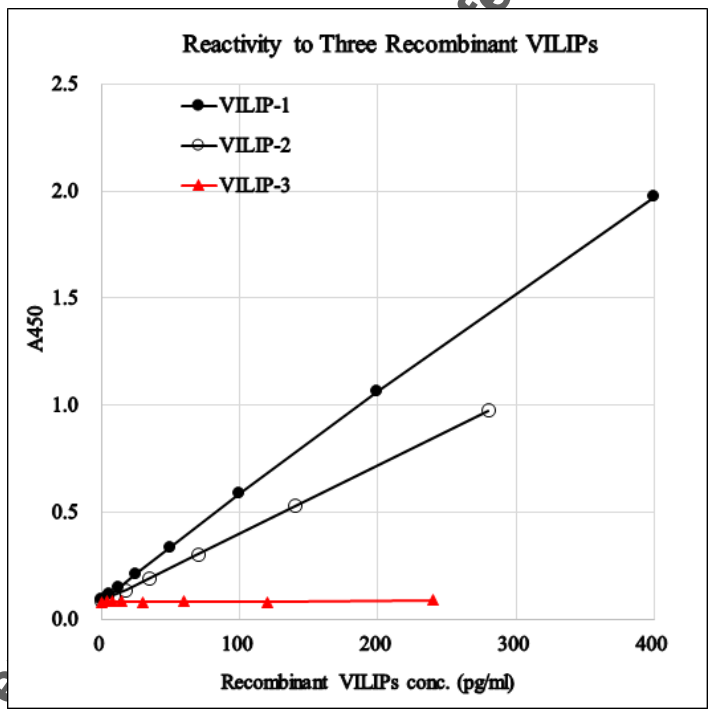
Three samples* were diluted with Dilution Buffer and assayed after dilution. The neat sample is set to 1. The results are summarized in the figure below.

*Sample: rat and mouse brain extract



4. Cross-reactivity of CircuLex VILIP-1/2 ELISA Kit

This kit shows approx. 70% cross-reactivity with VILIP-2 and no cross-reactivity with VILIP3.



Example of Test Results

Fig.1 VILIP-1/2 concentrations in rat and mouse brain extracts

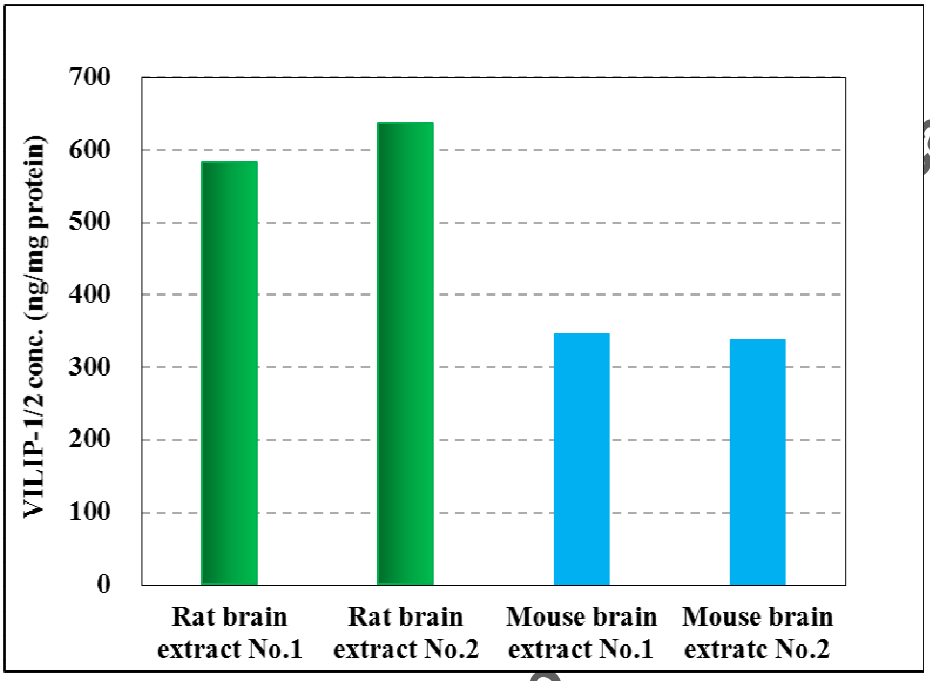
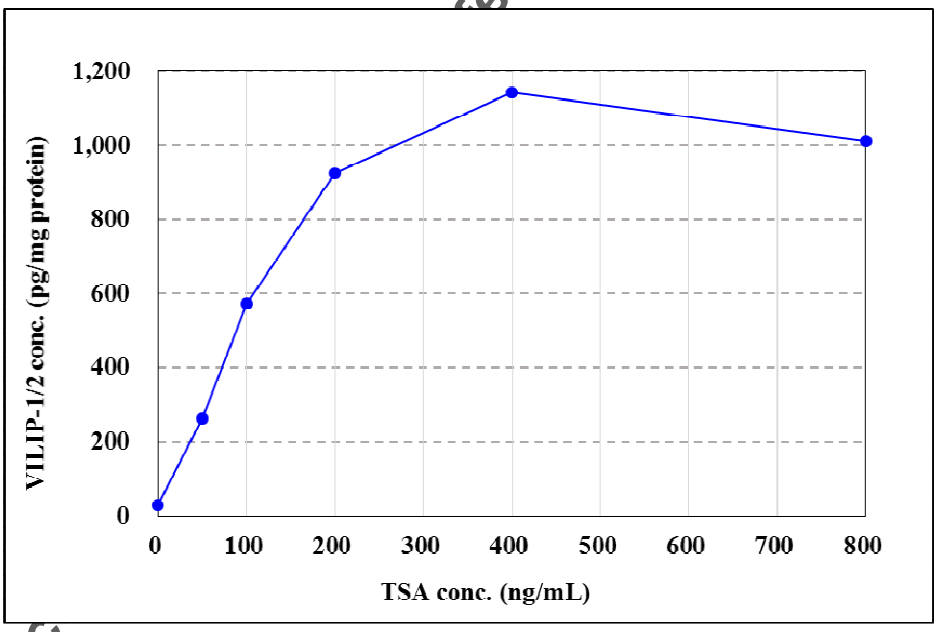


Fig.2 Induction of VILIP-1/2 expression by treatment with Trichostatin A in A549 cell line



References

1. Braunewell KH, Brackmann M, Schaupp M, Spilker C, Anand R, Gundelfinger ED.; Intracellular neuronal calcium sensor (NCS) protein VILIP-1 modulates cGMP signalling pathways in transfected neural cells and cerebellar granule neurones.
J Neurochem. 2001; 78(6): 1277-86.

2. Laterza OF, Modur VR, Crimmins DL, Olander JV, Landt Y, Lee JM, Ladenson JH.; Identification of novel brain biomarkers.
Clin Chem 2006; 52: 1713–21.

3. Mathisen PM, Johnson JM, Kawczak JA, Tuohy VK.; Visinin-like protein (VILIP) is a neuron-specific calcium-dependent double-stranded RNA-binding protein.
J Biol Chem. 1999; 274(44): 31571-6.

4. Kiyama H, Takami K, Hatakenaka S, Nomura I, Tohyama M, Miki N.; Localization of chick retinal 24,000 dalton protein (visinin)-like immunoreactivity in the rat lower brain stem.
Neuroscience 1985; 14: 547–56.

5. Takami K, Kiyama H, Hatakenaya S, Tohyama M, Miki N.; Localization of chick retinal visinin-like immunoreactivity in the rat forebrain and diencephalon.
Neuroscience 1985; 15: 667–75.

6. Paterlini M, Revilla V, Grant AL, Wisden W.; Expression of the neuronal calcium sensor protein family in the rat brain.
Neuroscience 2000; 99: 205–16.

7. McGinnis JF, Stepanik PL, Baehr W, Subbaraya I, Lerious V.; Cloning and sequencing of the 23 kDa mouse photoreceptor cell-specific protein.
FEBS Lett 1992; 302: 172–6.

8. Dai FF, Zhang Y, Kang Y, Wang Q, Gaisano HY, Braunewell KH, Chan CB, Wheeler MB.; The neuronal Ca2+ sensor protein visinin-like protein-1 is expressed in pancreatic islets and regulates insulin secretion.
J Biol Chem. 2006; 281: 21942-53.

9. Stejskal D, Sporova L, Svestak M, Karpisek M.; Determination of serum visinin like protein-1 and its potential for the diagnosis of brain injury due to the stroke: a pilot study.
Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2011; 155: 263-8.

10. Kester MI, Teunissen CE, Sutphen C, Herries EM, Ladenson JH, Xiong C, Scheltens P, van der Flier WM, Morris JC, Holtzman DM, Fagan AM: Cerebrospinal fluid VILIP-1 and YKL-40, candidate biomarkers to diagnose, predict and monitor Alzheimer's disease in a memory clinic cohort.
Alzheimers Res Ther. 2015; 7(1):59

Related Products

*** CircuLex Human UCHL1 ELISA Kit: Cat# CY-8092**
*** CircuLex Mouse UCHL1 ELISA Kit: Cat# CY-8093**
*** CircuLex Human 14-3-3 Gamma ELISA Kit: Cat# CY-8082**
*** CircuLex Human YKL-40 ELISA Kit: Cat# CY-8088**
*** CircuLex VILIP-1/2 ELISA Kit: Cat# CY-8106**

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