# VIP ELISA Kit

Enzyme Immunoassay for the quantification of VIP in Human, Mouse, Rat, Guinea pig, Pig, Sheep serum and plasma (EDTA) samples

Catalog number: ARG81246

Package: 96 wells

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## INTRODUCTION

VIP (vasoactive intestinal peptide) belongs to the glucagon family. It stimulates myocardial contractility, causes vasodilation, increases glycogenolysis, lowers arterial blood pressure and relaxes the smooth muscle of trachea, stomach and gall bladder. The protein also acts as an antimicrobial peptide with antibacterial and antifungal activity. Alternative splicing occurs at this locus and two transcript variants encoding distinct isoforms have been identified. [provided by RefSeq, Nov 2014]

VIP causes vasodilation, lowers arterial blood pressure, stimulates myocardial contractility, increases glycogenolysis and relaxes the smooth muscle of trachea, stomach and gall bladder.

PHM and PHV also cause vasodilation. PHM-27 is a potent agonist of the calcitonin receptor CALCR, with similar efficacy as calcitonin. [UniProt]

## PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification VIP in Human, Mouse, Rat, Guinea pig, Pig, Sheep serum and plasma samples. This assay employs the competitive quantitative enzyme immunoassay technique. A secondary antibody has been pre-coated onto a microtiter plate. The secondary antibody can bind to the Fc fragment of the primary antibody which recognizes VIP. The primary antibodies in the kit will be competitively bound by both biotinylated VIP peptides and VIP peptides in standards or targeted VIP peptides in samples. The wells are washed and then incubated with Streptavidin-HRP reagent. The biotinylated peptide interacts with streptavidin-horseradish peroxidase to form a complex. After washing away any unbound antibody conjugate, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of VIP present in the samples. The color development is stopped

by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm±2 nm. The concentration of VIP in the sample is then determined by comparing the O.D of samples to the standard curve.

## MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

| Component | Quantity | Storage information |
|---|---|---|
| Secondary antibody coated microplate | 12 x 8 wells | 4°C |
| 20X Wash Buffer | 50 ml | 4°C |
| Primary antibody | 1 vial | 4°C |
| Biotinylated peptide | 1 vial | 4°C |
| Standard | 1 vial | 4°C |
| Streptavidin-HRP complex | 30 µl | 4°C |
| TMB substrate | 12 ml (Ready-to-use) | 4°C (Protect from light) |
| STOP solution (2N HCl) | 15 ml (Ready-to-use) | 4°C |
| Positive Controls acceptable range: 0.1-0.3 ng/ml | 2 vial | 4°C |
| Plate sealer | 3 pieces | Room Temperature |

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Microplate shaker (300-400rpm)
- Automated microplate washer (optional)

## TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times (Do not freeze).
- Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lypholized material is at the bottom of the tube.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Unused microplate strips should be placed back in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-400 rpm is

recommended for all incubations.

- It is highly recommended that the standards, samples and controls be assayed in duplicates.

## SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1600 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 4°C at 1600 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Note: We recommended add Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.6 TIU or 100 µl of Aprotinin per mL of sample solution.

Peptide extraction –

1. It is recommended through peptide extraction protocol.
2. Mix an equal amount of Binding Buffer (1% trifluoroacetic acid (TFA), HPLC grade is recommended) with the plasma or serum samples and vortex the mixture. Centrifuge at 6,000-17,000 × g for 20 minutes at 4°C. Collect the supernatant.
3. Slowly equilibrate a C18 column (SEP-COLUMN containing 200 mg of C18)

by washing the C18 column with 1 ml Elution Buffer (60% acetonitrile, 1% TFA, and 39% distilled water, HPLC grade of acetonitrile and TFA are recommended).

4. Wash the C18 column with 3 ml of Binding Buffer three times.
5. Load the sample/Binding Buffer mixture solution from step 1 into the washed C18 column from step 3.
6. Wash the column slowly with Binding Buffer (3 ml, twice) and discard the wash.
7. Elute the peptide slowly with Elution Buffer (3 ml, once) and collect eluant into a polystyrene tube.
8. Evaporate eluant to dryness in a centrifugal concentrator or by a suitable substitute method to dryness the eluant. (Freeze-dry the resulting water/TFA solution to dryness)
9. Keep the dried extract at -20°C and perform the assay as soon as possible.
10. For normal subject extracted from 1 ml original plasma/serum, use 125 µl 1X Wash buffer to reconstitute the dried extract. Aliquot 50 µl into two designated assay wells (25 µl is left over). The concentration factor in this case is 8. (1 ml / 125 ul = 8). The original plasma peptide level is 1/8 of the level of final extracted plasma.

    e.g. If the level of the final extracted plasma is 100 pg/ml, then the total level of peptide in the original plasma = (100 pg/ml) / 8 = 12.5 pg/ml. After performing assay, if the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the samples accordingly.

Drying Sample After Extraction:

A combination of a centrifugal concentrator (i.e. Speedvac) and a lyophilizer (freeze-dryer) produces the best results for drying the sample after extraction. First, use a Speedvac to dry sample for approximately 15 minutes to remove the organic layer. Then snap-freeze the remaining sample, and freeze-dry overnight using a lyophilizer. This two-step procedure produces a more consistent fluffy powder that is easier to rehydrate than a sample dried only with a centrifugal concentrator. However, if a centrifugal concentrator is not accessible, freeze-drying overnight using a lyophilizer will be sufficient.

## REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash buffer. Keep 1X Wash Buffer at 4°C. If crystals appear in 20X Wash Buffer, warm the buffer in warm water bath (not higher than 50°C) for 30 minutes or until crystals disappear. Mix well before use.
- Primary antibody: Reconstitute the Primary antibody vial with 5 ml of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C.
- Biotinylated peptide: Reconstitute the Biotinylated peptide vial with 5 ml of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C.
- Positive control: Centrifuge and reconstitute the Positive control vial with 200 µl of 1X Wash Buffer. Allow it to sit for 5 minutes to completely dissolve, mix well before use. Keep rehydrated solution at 4°C.

(acceptable range: 0.1-0.3 ng/ml)

- **Streptavidin-HRP complex:** Centrifuge Streptavidin-HRP complex briefly and add **12µl** of Streptavidin-HRP to **12ml** of 1X wash buffer to make a Streptavidin-HRP solution. Vortex thoroughly.
- **Standard peptide**: Centrifuge and dilute the standard with **1 ml** of 1X Wash buffer and vortex. The concentration of this stock solution is **1000 ng/ml**. Allow the solution to sit for at least 10 minutes at room temperature to completely dissolve. Dilute peptide standard solutions with 1X Wash buffer to **25 ng/ml**, **5 ng/ml**, **1 ng/ml**, **1 ng/ml**, **0.2 ng/ml** and **0.04 ng/ml** as follows:

The example of the dilution of standards

| Standard No. | Standard Conc. (ng/ml) | 1X Wash Buffer (µl) | Standard (µl) |
|---|---|---|---|
| Stock | 1000 | - | - |
| S1 | 25 | 975 | 25 µl of Stock |
| S2 | 5 | 800 | 200 µl of S1 |
| S3 | 1 | 800 | 200 µl of S2 |
| S4 | 0.2 | 800 | 200 µl of S3 |
| S5 | 0.04 | 800 | 200 µl of S4 |
| S0 (Total binding) | 0 | 800 | 0 |

## ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-23°C) 30 minutes before use. Standards, samples and blank should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl of 1X Wash Buffer** as Total Binding (S0, zero standard). Two empty wells should be left as blank.
3. Add **50 µl of prediluted peptide standards** (add from S5 to S1), **50 µl** of **positive controls** or **50 µl** of **samples** into corresponding wells. It is advisable to assay each condition in duplicates.
4. Add **25 µl of primary antibody** into each well **except the Blank wells**.
5. Add **25 µl of Biotinylated peptide** into each well **except the Blank wells**. It is not recommended to use a multi-channel pipette to load the primary antibody and biotinylated peptide.
6. Seal the microtiter plate with plate sealer. Incubate for **2 hours at RT.** Orbital shaking at 300-400 rpm is recommended.
7. Mix and centrifuge Streptavidin-HRP concentrate vial (3,000-5,000 rpm for 5 seconds) before use. Pipette 12 µl of Streptavidin-HRP concentrate into 12 ml of 1X Wash Buffer to make Streptavidin-HRP working solution. Vortex thoroughly. Prepare freshly.
8. Remove sealer from plate.
9. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with **1× Wash Buffer (350 µl)** using a

squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

10. Add **100 µl** of **diluted (1X) Streptavidin-HRP** working solution into **each well**.
11. Reseal the plate with sealer. Incubate for **1 hour at RT.** Orbital shaking at 300-400 rpm is recommended.
12. Remove sealer from plate. **Wash** as according to step 9.
13. Add **100 µl** of **TMB substrate** solution into **each well**.
14. Reseal the plate with sealer. Incubate for **1 hour at RT in dark** Orbital shaking at 300-400 rpm is recommended.
15. Remove sealer from plate. (**DO NOT** wash or discard the contents of the wells)
16. Add **100 µl** of **STOP solution (2N HCl)** into **each wells** to stop the reaction. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
17. **Read the OD** with a microplate reader **at 450 nm immediately**. It is recommended that the wells be read within 20 minutes after adding the Stop Solution.

## CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

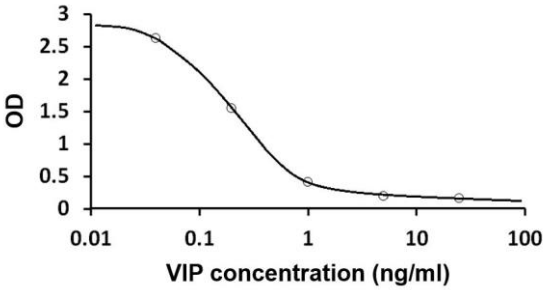
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors.

## EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.




## QUALITY ASSURANCE

### Sensitivity

The standard of VIP ranged from 0-25 ng/ml.

The mean MDD was 0.09 ng/ml.

### Linear Range

0.09 – 0.81 ng/ml

### Precision:

Intra-assay: < 10%

Inter-assay: < 15%

## Cross Reactivity

The cross reactivity ratio of the tested peptide as the table:

| Peptide | Cross Reactivity (%) |
|---|---|
| VIP (Human, Mouse, Rat, Porcine, Ovine) | 100 |
| VIP (Guinea Pig) | 100 |
| VIP (10-28) (Human, Porcine, Rat) | 34.6 |
| VIP (Chicken) | 28 |
| PACAP-27-NH2 (Human, Rat, Ovine) | <0.02 |
| VIP (1-12) (Human, Porcine, Rat) | 0 |
| PHM-27 (Human) | 0 |
| Substance P (Human, Mouse, Rat) | 0 |
| Endothelin-1 (Human, Mouse, Rat, Porcine, Canine, Bovine) | 0 |
| Secretin (Porcine) | 0 |
| Glucagon (Human, Mouse, Rat, Porcine, Canine, Bovine) | 0 |
| Galanin (Rat) | 0 |
| Somatostatin (Human, Mouse, Rat, Porcine) | 0 |
| PACAP-38 (Human, Rat, Ovine) | 0 |