# TECHNICAL MANUAL

# Human VIP (Vasoactive Intestinal Peptide) ELISA Kit

- **SKU CODE:** HUES03058
- **SIZE:** 48T/96T
- **DETECTION PRINCIPLE:** Competitive
- **RUO:** Research-Use-Only

Revised: 02/2026

# Human VIP (Vasoactive Intestinal Peptide) ELISA Kit

*Please read entire manual carefully before starting experiment. DO NOT mix reagents and use reagents from different kits or batches to prevent assay failure.*

## Table of Contents

# 1. Key Features

**Detection Method:**

Competitive (Antigen Coated)

**Sample Type:**

Serum, Plasma And Other Biological Fluids

**Reactivity:**

Human

**Range:**

7.81-500 pg/mL

**Sensitivity:**

4.69 pg/mL

# 2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit and/or components as described in section 4. Date of expiration is on the ELISA Box label.

# 3. Product Description

The Assay Genie Human VIP (Vasoactive Intestinal Peptide) ELISA Kit is a highly sensitive assay for the quantitative measurement of a Human VIP in the following samples: Serum, Plasma And Other Biological Fluids.

This kit is based on the principle of a competitive enzyme-linked immunosorbent assay (ELISA) principle, where the microtiter wells are pre-coated with Human VIP. In this setup, the Human VIP present in the sample competes with the immobilized antigen for binding to a limited amount of biotin-labelled antibody.

When the sample and the biotin-labelled antibody are added, higher concentrations of Human VIP in the sample will block more antibody binding to the coated antigen.

After washing away unbound materials, HRP-Streptavidin is added, which binds to the biotin-labelled antibodies on the well. The Substrate Reagent is then added, producing a colour reaction. The colour intensity is inversely proportional to the concentration of Human VIP in the sample, higher sample concentrations result in a weaker colour signal (lower OD at 450 nm).

The concentration of Human VIP is determined by comparing sample OD values to the standard curve.

**This dual function kit includes validated Bradford Reagent to quantify total protein concentration for accurate sample normalization.**

# 4. Kit Contents

| No | Component Name | Specifications | Storage |
|---|---|---|---|
| **1** | Micro ELISA Plate (Dismountable) | 96T: 8 wells ×12 strips \| 48T: 8 wells ×6 strips \| 24T: 8 wells ×3 strips \| 96T*5: 5 plates, 96T | -20°C, 12 months |
| **2** | Reference Standard | 96T: 2 vials \| 48T/24T: 1 vial \| 96T*5: 10 vials | -20°C, 12 months |
| **3** | Concentrated Biotinylated Detection Ab(100×) | 96T: 1 vial, 120 µL \| 48T/24T: 1 vial, 60 µL \| 96T*5: 5 vials, 120 µL | -20°C, 12 months |
| **4** | Concentrated HRP Conjugate (100×) | 96T: 1 vial, 120 µL \| 48T/24T: 1 vial, 60 µL \| 96T*5: 5 vials, 120 µL | -20°C (Protect from light), 12 months |
| **5** | Reference Standard & Sample Diluent | 96T/48T/24T: 1 vial, 20 mL \| 96T*5: 5 vials, 20 mL | 2–8°C, 12 months |
| **6** | Biotinylated Detection Ab Diluent | 96T/48T/24T: 1 vial, 14 mL \| 96T*5: 5 vials, 14 mL | 2–8°C, 12 months |
| **7** | HRP Conjugate Diluent | 96T/48T/24T: 1 vial, 14 mL \| 96T*5: 5 vials, 14 mL | 2–8°C, 12 months |
| **8** | Concentrated Wash Buffer(25×) | 96T/48T/24T: 1 vial, 30 mL \| 96T*5: 5 vials, 30 mL | 2–8°C, 12 months |
| **9** | Substrate Reagent | 96T/48T/24T: 1 vial, 10 mL \| 96T*5: 5 vials, 10 mL | 2–8°C (Protect from light) |
| **10** | Stop Solution | 96T/48T/24T: 1 vial, 10 mL \| 96T*5: 5 vials, 10 mL | 2–8°C |
| **11** | Plate Sealer | 96T/48T/24T: 5 pieces \| 96T*5: 25 pieces | 2–8°C |
| **12** | Technical Manual | 1 copy | - |
| **13** | Certificate of Analysis | 1 copy | - |
| **14** | Bradford Reagent | 96T: 1 vial \| 48T: 1 vial | 4°C |

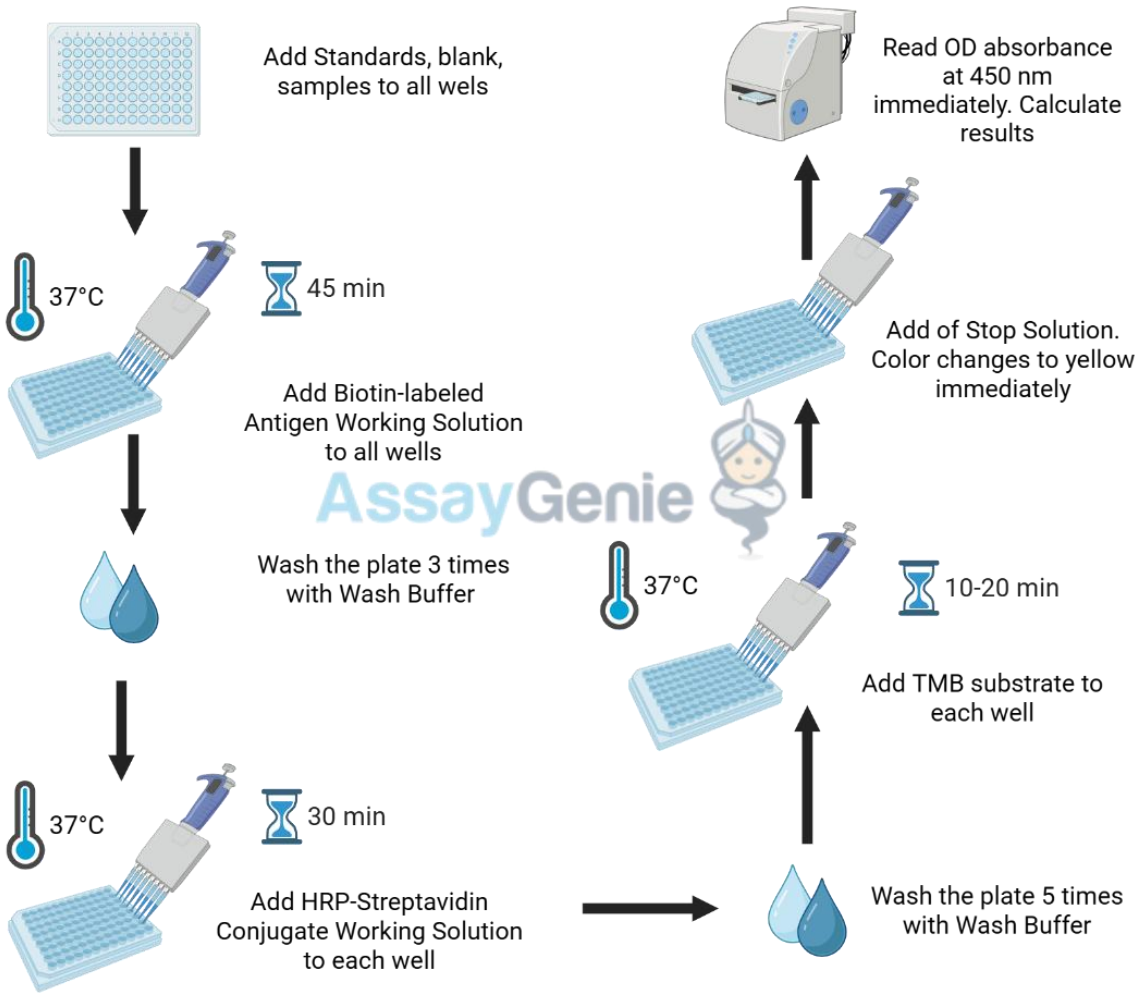
### Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

## 5. Precautions

1. This kit is for research purposes only and not for diagnostics or therapeutic uses.
2. To identify the concentration of your target, a pilot experiment using standards and a small number of samples is recommended.
3. Store all components as listed in this manual. Do not use the ELISA Kit after its expiration date.
1. Ensure unopened and unused plate are kept dry to avoid contamination.
4. Before using the kit, centrifuge tubes to spin down standard & antibodies.
5. Avoid light for storage of TMB reagents.
6. Wash steps are critical for the success of the assay, deviations from wash steps may cause false positives and result in a high background.
7. Duplicate wells are recommended for both standard and sample testing.
8. Do not let the microplate wells dry during the assay.
9. Do not reuse tips and tubes to avoid cross contamination.
10. Avoid using the reagents from different batches together.

# 6. Assay Summary



# 7. Sample Preparation

The procedures outlined in this document are provided as general recommendations for sample preparation in ELISA assays. Due to the variability of biological samples and specific assay requirements, users are advised to optimize protocols based on their own experimental conditions.

***Note**: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.*

## General Considerations

To prevent denaturation or degradation of target proteins, it is recommended to process samples promptly and store them under appropriate conditions.

- **Storage Conditions**:
  - **Short-term**: 2-8 °C for up to 5 days.
  - **Medium-term**: -20 °C for up to 6 months.
  - **Long-term**: -80 °C or cryopreservation in liquid nitrogen.
- **Thawing Protocol**: Frozen samples should be thawed rapidly in a 15-25 °C water bath to minimize ice crystal-induced damage. Thawed samples can be analyzed immediately or stored temporarily at 2-8 °C.
- **Freeze-Thaw Cycles**: Repeated freeze-thaw cycles should be strictly avoided due to their detrimental effect on protein stability.

### A. Blood-Derived Samples

- **Serum:** Allow whole blood to coagulate at room temperature (2 h) or 2-8 °C overnight. Centrifuge at 1000 × g for 20 min and collect the supernatant. Store or use immediately.
- **Plasma:** Collect in anticoagulant tubes (EDTA, citrate, or heparin), mix gently, and centrifuge within 30 min at 1000 × g, 2-8 °C for 15 min. Store or assay as needed.

**B. Tissue Homogenates**

Tissue samples should be homogenized prior to use. Avoid buffers containing NP-40, Triton X-100, or DTT, as these strongly inhibit the assay. We recommend using 50 mM Tris + 0.9% NaCl + 0.1% SDS, pH 7.3.

The recommended protocol is as follows:

- **Sample Collection and Washing**
    - Place the target tissue on ice.
    - Rinse the tissue with pre-cooled PBS buffer (0.01 M, pH 7.4) to remove residual blood.
    - Weigh the tissue for further processing.
- **Homogenization**
    - Homogenize the tissue on ice using an appropriate lysis buffer.
    - The lysate volume should correspond to the tissue weight; typically, 9 mL PBS is used per 1 g of tissue. It is recommended to add protease inhibitors to the PBS (e.g., 1 mM PMSF). ***Note:*** *PBS buffer or mild RIPA lysis buffer can be used for homogenization. When using RIPA, adjust pH to 7.3.*
- **Cell Disruption**
    - Further disrupt the tissue using ultrasonic homogenization or freeze–thaw cycles.
        - Ultrasonic homogenization: Keep samples on an ice bath during sonication to avoid overheating.
        - Freeze–thaw cycles*:* Repeat twice for effective lysis.
- **Centrifugation and Storage**
    - Centrifuge the homogenate at 5000 × g for 5 minutes.
    - Collect the supernatant for immediate analysis, or aliquot and store at –20°C or –80°C for future assays.

- **Protein Concentration Measurement**
    - Determine total protein concentration using the Bradford Reagent included in this kit.
    - For ELISA assays, the total protein concentration should generally be 1–3 mg/mL.
    - Tissues with high endogenous peroxidase levels (e.g., liver, kidney, pancreas) may react with TMB substrate, causing false positives. If this occurs, treat samples with 1% $H_2O_2$ for 15 minutes before repeating the assay.

***Note:*** *Liver, kidney, and pancreas samples often contain high levels of endogenous peroxidase, which may react with the chromogenic substrate at elevated sample concentrations, potentially resulting in false positive signals.*

*If analysis of these tissues is required, a gradient dilution assay is recommended. A proportional decrease in signal with increasing dilution typically indicates minimal interference and supports the accuracy of the results.*

*To further minimise potential interference, samples can be pre-treated with 1% hydrogen peroxide ($H_2O_2$) for 15 minutes prior to testing. To prepare the treatment solution, add 1 µl of pure $H_2O_2$ to 100 µl of sample (1% v/v).*

**C. Cell Culture Supernatant**

Centrifuge the sample at 2500 rpm for 5 minutes at 2–8°C. Carefully collect the clarified cell culture supernatant for immediate analysis, or aliquot and store it at –80°C for future assays.

**D. Cell Lysates**

- **Suspension Cell Lysate:** Centrifuge the cell suspension at 2500 rpm for 5 minutes at 2–8°C and collect the cell pellet. Wash the pellet with pre-cooled PBS (0.01 M, pH 7.4) and mix gently. Repeat centrifugation and discard the supernatant. Add 0.5–1 mL of cell lysis buffer containing an appropriate protease

inhibitor (e.g., PMSF, final concentration: 1 mM). Lyse the cells on ice for 30–60 minutes or disrupt them using ultrasonic homogenization.

- **Adherent Cell Lysate:** Remove the supernatant and wash the cells three times with pre-cooled PBS. Add 0.5–1 mL of cell lysis buffer supplemented with an appropriate protease inhibitor (e.g., PMSF at a final concentration of 1 mmol/L). Scrape the adherent cells using a cell scraper and transfer the cell suspension to a centrifuge tube. Lyse the cells on ice for 30–60 minutes or disrupt the cells by ultrasonic treatment.

Follow next steps for protein extraction and supernatant collection:

- **Protein Release and DNA Disruption**
    - During lysis, pipette gently or intermittently shake the tube to enhance protein extraction.
    - Mucilaginous material formed during lysis is DNA, which can be broken down by ultrasonic disruption (3–5 mm probe, 150–300 W, 3–5 seconds per cycle, with 30-second intervals for 1–2 minutes total).
- **Supernatant collection**
    - After lysis or ultrasonic treatment, centrifuge the lysate at 10,000 rpm for 10 minutes at 2–8°C. Collect the supernatant for immediate use or aliquot and store at –80°C for future assays.

***Notes:*** *Refer to the "Tissue Sample Notes" for additional buffer and inhibitor recommendations.*

**E. Other Sample Types**

For more information about how to process other sample types, (e.g., body fluids, breast milk & more), please contact our Tech Support Team at techsupport@assaygenie.com.

## 7.1. Protein Quantification (Optional)

To quantify total protein levels, use the Bradford Reagent included in this kit. Visit https://www.assaygenie.com/bradford-protein-assay-protocol/ to view the full protocol.

# 8. Reagent Preparation

### Manual Washing

Discard the solution in the plate without touching the side of the wells. Clap the plate on absorbent filter paper or other absorbent material. Fill each well completely with 350 µl wash buffer and soak for 1 to 2 mins, then aspirate contents from the plate, and clap the plate on absorbent filter paper or other absorbent material. Repeat this procedure for the designated number of washes.

### Automated Washing

Aspirate all wells, then wash plate with 350 µl wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter paper or other absorbent material. It is recommended that the washer is set for a soaking time of 1 minute.

***Note:*** *Set the height of the needles; be sure the fluid can be taken up completely.*

### Sample Dilution Guidelines

Determine the concentration of the target protein in the test sample and then select the optimal dilution factor to ensure the target protein concentration falls within the optimal detection range of the kit. Dilute the samples with the dilution buffer provided with the kit. Several dilution tests may be required to achieve the optimal results. The test samples must be well mixed with the dilution buffer. Standard and sample dilution should be performed before starting the experiment.

***Note:*** *Dilution may be necessary to minimize matrix effects. However, if the target concentration in the sample is very low, the pre-treated sample can be added directly to the assay without dilution.*

## Reagent Preparation

Bring all reagents and samples to room temperature 20 minutes before use (18 - 25°C). For repeated assays, please use only strips and standards required and store remaining reagents at the appropriate temperatures.

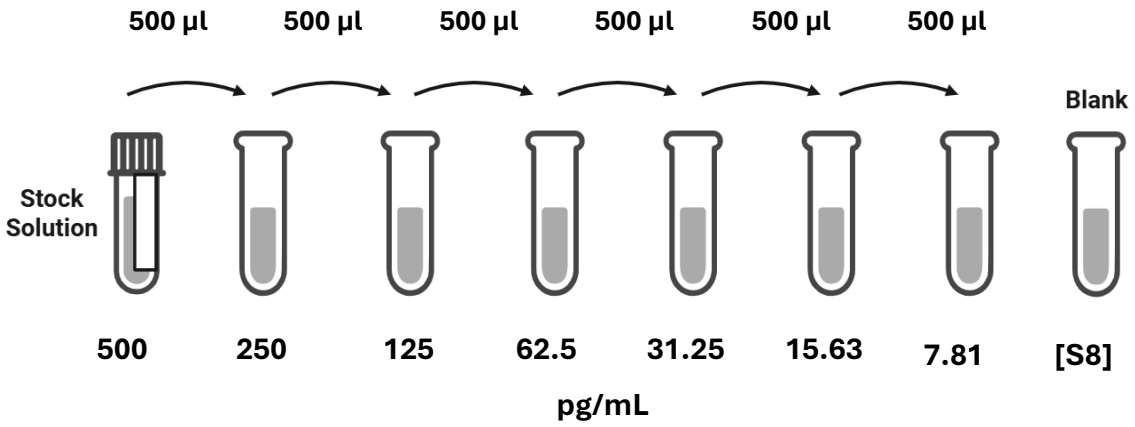
**A. Wash Buffer:**

Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer (recommended resistivity of ultrapure water is 18MΩ). Store unused solution at 4°C. If crystals have formed in the concentrate, warm at 40°C in water bath (Heating temperature should not exceed 50°C) and mix gently until crystals have completely dissolved. The solution should be cooled to room temperature before use.

**B. Standard Dilution:**

1. Centrifuge the standard tube for 1 min at 10,000 x g.
2. Add 1 of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 500 pg/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 500, 250, 125 62.5, 31.25, 15.63, 7.81 and 0 pg/mL. ***Note:*** *The final tube serves as the blank and should not receive any solution transferred from the preceding tube*.
3. Take 7 Eppendorf tubes add 500 µL of Reference Standard & Sample Diluent to each tube. Pipette 500 µL of the 500 pg/mL working solution to the first tube and mix up to produce a 250 pg/mL working solution. Pipette 500µL of the solution from the former

tube into the latter one according to these steps. The illustration below is for reference.




***Note:*** *The reconstituted standard solution should be aliquoted and stored at −20 °C. It must be used within 2 weeks, and repeated freeze–thaw cycles should be avoided. Gradient-diluted working standards should be freshly prepared immediately before use.*

**C. Preparation of Biotinylated Detection Ab working solution:**

Prepare before starting the experiment.

1. Calculate required total volume of the working solution: 50 µl /well x quantity of wells.
***Note:*** *It is advisable to prepare an amount marginally exceeding the calculated requirement.*

2. Centrifuge for 1min at 800 x g in low speed and bring down the concentrated biotin-labelled antibody to the bottom of tube.

3. Dilute the biotinylated detection antibody 100x to 1x working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection: Biotinylated Detection Ab Diluent= 1: 99).

**D. Preparation of HRP-Conjugate Working Solution:**

Prepare before starting the experiment.

1. Calculate the total volume of the working solution: 100 µl / well x quantity of wells. ***Note:*** *It is advisable to prepare an amount marginally exceeding the calculated requirement*.

2. Centrifuge for 1 min at 800 x g in low speed and bring down the concentrated SABC to the bottom of the tube.

3. Dilute the 100 x Concentrated HRP Conjugate to 1 x working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

# 9. Assay Procedure

1. **Plate Setup:** Designate standard wells, test sample wells, and control (blank) wells on the pre-coated plate, and record their positions. It is recommended to measure each standard and sample in duplicate. ***Note:*** *Determine the optimal dilution ratio for samples through preliminary experiments or by following technical support recommendations*.
2. **Standard, Samples & Control Loading:** Aliquot 50 µl of standard working solution or samples and controls into the designated wells**.**
3. **Addition of Biotin-Labeled Antibody:** Immediately add 50 µL of the biotin-labeled antibody working solution to each well (standards, samples, and blanks). Cover the plate with the provided plate sealer, gently tap to ensure complete mixing, and incubate for 45 minutes at 37°C. ***Note:*** *Solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.*

4. **Washing:** Decant the solution from each well and add 350 µL of wash buffer to each well. Let it soak for 1 minute, then aspirate or decant the solution and blot the plate on clean absorbent paper. Repeat this washing step 3 times. ***Note:*** *A microplate washer may be used for this and subsequent wash steps. Do not allow the wells to dry after washing*.
5. **HRP Conjugate Working Solution Addition:** Add 100 µL of HRP Conjugate working solution to each well. Cover the plate and incubate at 37°C for 30 minutes.
6. **Washing:** Decant the solution from each well, repeat the wash process for 5 times as conducted in step 4.
7. **Substrate Reagent:** Add 90 µL of Substrate Reagent solution to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light. ***Note:*** *The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement*.
8. **Stop Solution:** Add 50 µL of Stop Solution to each well and mix thoroughly. The colour will change to yellow immediately.
9. **OD Measurement:** Read the optical density (OD) at 450 nm in a microplate reader immediately after adding the Stop Solution.

# 10. Data Analysis

This assay uses a competitive inhibition enzyme immunoassay format; therefore, the assay signal intensity is inversely proportional to the concentration of Human VIP in the sample.

Average the duplicate absorbance readings for each standard, control, and sample. Generate a standard curve by plotting Human VIP concentration on the y-axis against absorbance on the x-axis. Determine the best-fit line through the standard points using regression analysis.

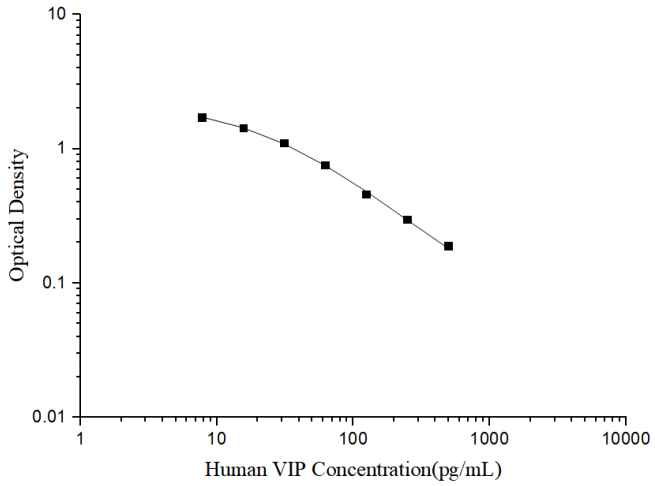
For diluted samples, multiply the concentration obtained from the standard curve by the corresponding dilution factor to calculate the final concentration. Curve fitting and data analysis may be performed using appropriate software (e.g., CurveExpert).

***Note:*** *If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.*

# 11. Typical Data

### Standard Curve

Results of a typical standard run of an ELISA kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.



## Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and high levels were tested 20 times on one plate, (n= replicate).

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and high level were tested on 3 different plates, 20 replicates in each plate.

| | Intra-assay Precision | | | Inter-assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | **20** | **20** | **20** | **20** | **20** | **20** |
| **Mean pg/mL** | 27.57 | 77.47 | 196.79 | 30.14 | 77.54 | 215.13 |
| **Standard deviation** | 1.54 | 3.66 | 13.5 | 2.2 | 6.4 | 16.41 |
| **CV (%)** | 5.57 | 4.72 | 6.86 | 7.29 | 8.25 | 7.63 |

## Recovery

Matrices listed below were spiked with a certain level of Human VIP and the recovery rates were calculated by comparing the measured value to the expected amount of Human VIP in the samples.

| Sample Type | Range (%) | Average Recovery (%) |
|---|---|---|
| **Serum (n=8)** | 92-104 | 98 |
| **EDTA Plasma (n=8)** | 90-103 | 97 |
| **Cell Culture Media (n=8)** | 86-102 | 93 |

**Linearity**

The linearity of the kit was assayed by testing the samples spiked with appropriate concentration of Human VIP and their serial dilutions.

| | | Serum (n=5) | EDTA Plasma (n=5) | Cell Culture Media (n=5) |
|---|---|---|---|---|
| 1:2 | **Range (%)** | 89-100 | 93-107 | 92-107 |
| | **Average (%)** | 94 | 98 | 99 |
| 1:4 | **Range (%)** | 83-97 | 92-104 | 99-114 |
| | **Average (%)** | 90 | 97 | 104 |
| 1:8 | **Range (%)** | 87-100 | 93-105 | 97-112 |
| | **Average (%)** | 94 | 98 | 103 |
| 1:16 | **Range (%)** | 83-96 | 86-102 | 95-111 |
| | **Average (%)** | 90 | 93 | 102 |

# 12. ELISA Troubleshooting

| Problem | Possible Causes | Solutions |
|---|---|---|
| **Standard curve without signal** | Incorrect reagent order; Mixed components from different kits; Missing reagents. | Ensure correct reagent order and use components from the same kit. Verify all reagents are added. |
| **Overflow OD** | Mixed components from different kits; Over-concentrated working solution. | Use correct components and prepare solutions at recommended concentrations. |
| **Poor standard curve** | Incorrect curve fitting model. | Try alternative curve fitting models. |
| **Samples without signal** | Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw. | Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage. |
| **High CV%** | Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency. | Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes. |
| **Low standard signal** | Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells. | Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying. |
| **Slow colour development** | TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing. | Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times. |
| **High background** | Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light. | Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark. |

## Notes:

## Notes:

## Notes:

**Assay Genie 100% money-back guarantee!**

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

**Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.**