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619-302-0129

Human IL-1β ELISA Kit

E140706CTG-10

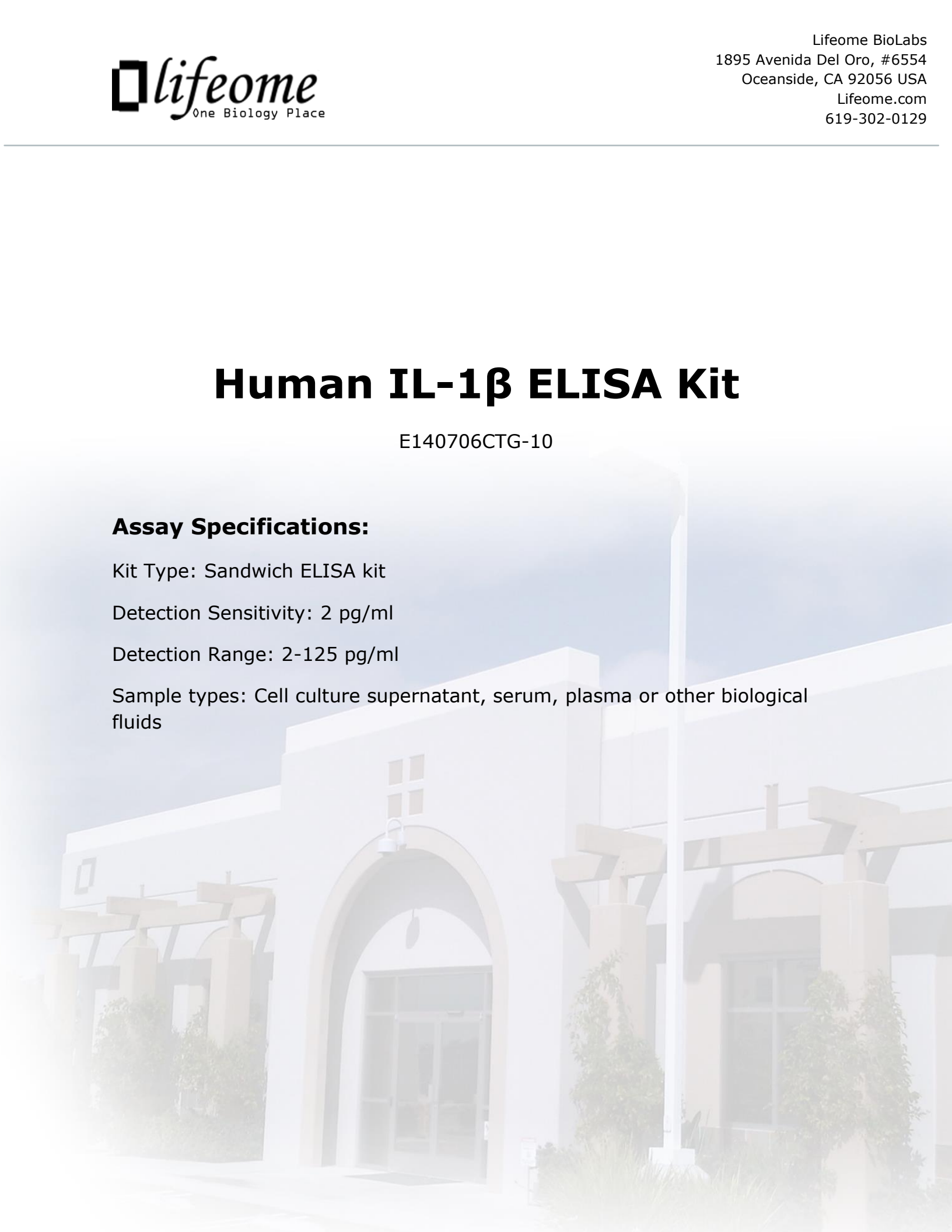
Assay Specifications:

Kit Type: Sandwich ELISA kit

Detection Sensitivity: 2 pg/ml

Detection Range: 2-125 pg/ml

Sample types: Cell culture supernatant, serum, plasma or other biological fluids



Human IL-1β Elisa Kit

Catalog number: E140706CTG-10

Lifeome's IL-1β Elisa kit is a sandwich immunoassay kit for the accurate quantification of natural and recombinant human IL-1β. This kit is designed for cost-effective and reliable quantification of human IL-1β in cell culture supernatant, serum, plasma or other biological fluids.

Introduction

IL-1 β refers to two proteins, IL-1α and IL-1β, which are the products of distinct genes, but are recognized by the same cell surface receptors. IL-1 binds to the cell surface type I and II IL-1 receptors. IL-1α, IL-1β, and IL-1ra can compete for binding to these receptors. However, only IL-1RI, not IL-1RII, is functional because IL-1RII lacks a cytoplasmic domain and is thus unable to transmit signals to downstream steps.

Test Principle

Lifeome's IL-1β kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A human IL-1β specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-1β binds to the immobilized capture antibody. Next, a biotinylated anti-human IL-1β detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IL-1β present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader

Reagents and Materials provided

1. Human IL-1β ELISA Detection Antibody (200µl, 200X)
2. Human IL-1β Standard (20ng)
3. Avidin-HRP (20µl, 1000X)
4. Substrate Solution F (10 ml)
5. Assay Diluent A (10 ml, 5X)
7. 96 MicroWell Plates coated with Human IL-1β monoclonal Antibody
8. Wash Buffer (20ml, 30×)
9. Stop buffer (10 ml)
10. Instruction Sheet, Lot-Specific Instruction/ Analysis Certificate

Materials required but not provided

• A microplate reader capable of measuring absorbance at 450 nm
• Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
• PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g Na2HPO4, 0.2 g KH2PO4, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered.
• Wash bottle or automated microplate washer
• Log-Log graph paper or software for data analysis
• Tubes to prepare standard dilutions

• Plate Shaker and Sealer
• Absorbent paper

Storage Information

• Store kit components at 4°C.
• After reconstitution of the lyophilized standard with 1X Assay Diluent A, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
• Prior to use, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions

Specimen Collection and Handling

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.
Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X g. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.
Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1,000 X g within 30 minutes of collection. Assay immediately or store plasma samples at < -20°C. Avoid repeated freeze/thaw cycles. Plasma specimens should be clear and non-hemolyzed.

Reagent Preparation

Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.

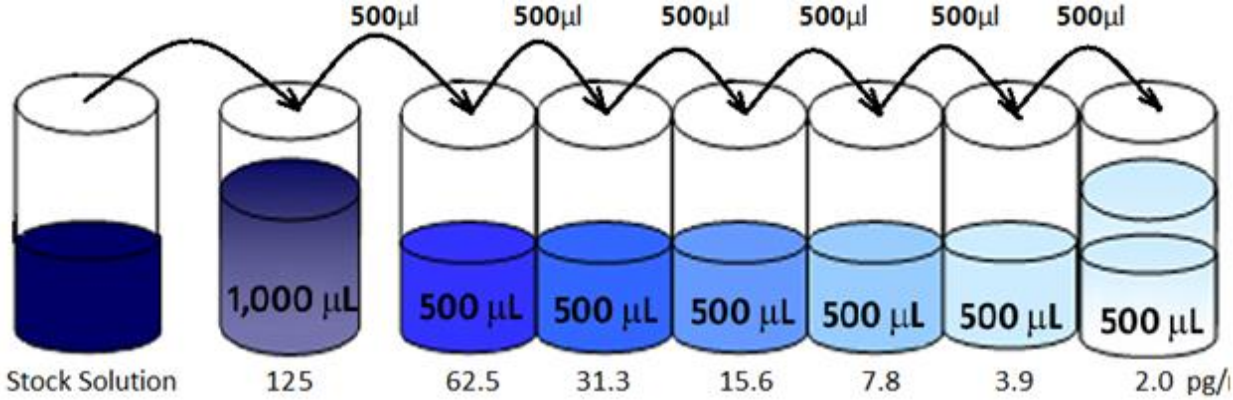
1. Dilute 5X Coating Buffer A to 1X with deionized water. For one plate, dilute 2.4 mL 5X Coating Buffer A in 9.6 mL deionized water.
2. Dilute 5X Assay Diluent A to 1X with PBS (pH 7.4). For 50 mL, dilute 10 mL 5X Assay Diluent A in 40 mL PBS. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.
3. Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard with 0.2 mL of 1X Assay Diluent A. Allow the reconstituted standard to sit for 15 minutes at room temperature, then vortex prior to making dilutions.
4. Prior to use, prepare 1,000 µL of the top standard at a concentration of 125 pg/mL from the stock solution in 1X Assay Diluent A (refer to Lot-Specific Instruction/Analysis Certificate).
5. Dilute the pre-titrated Biotinylated Detection Antibody 1:200 in 1X Assay Diluent A. For one plate, dilute 60 µL Detection Antibody in 11.94 mL 1XAssay Diluent A.
6. Dilute Avidin-HRP 1:1000 in 1X Assay Diluent A. For one plate, dilute 12 µL Avidin-HRP in 11.99 mL 1X Assay Diluent A.

Assay Procedure

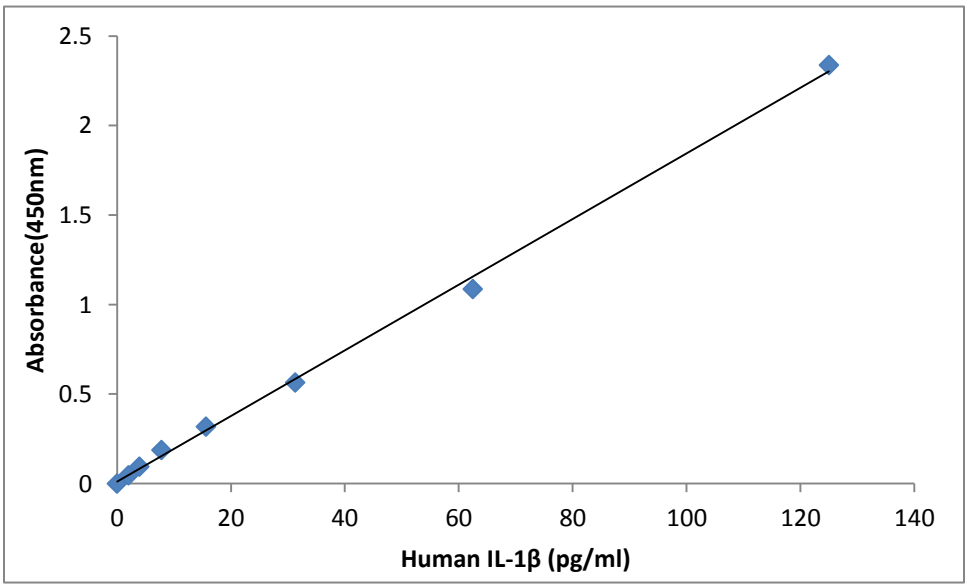
Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.

1. Prepare 1,000 µL of the top standard at 1000 pg/mL from stock solution in 1X Assay Diluent A (refer to Reagent Preparation). Perform six two-fold serial dilutions of the 125 pg/mL top standard with Assay Diluent A in separate tubes. After dilution, the human IL-1β standard

concentrations are 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, 15.6 pg/mL, 7.8 pg/mL, 3.9 pg/mL, and 2.0 pg/mL, respectively. Assay Diluent A serves as the zero standard (0 pg/mL).



2. Add 100 µL/well of standard dilutions or samples to the appropriate wells.
3. Seal plate and incubate at RT for 2 hours with shaking.
4. Wash plate 4 times with Wash Buffer.
5. Add 100 µL of diluted Detection Antibody solution to each well, seal plate and incubate at RT for 1 hour with shaking.
6. Wash plate 4 times with Wash Buffer.
7. Add 100 µL of diluted Avidin-HRP solution to each well.
8. Seal plate and incubate at RT for 30 minutes with shaking.
9. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
10. Add 100 µL of Substrate Solution F to each well and incubate in the dark for 20 minutes*. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
11. Stop reaction by adding 100 µL of Stop Solution to each well. Positive wells should turn from blue to yellow.
12. Read absorbance at 450 nm within 30 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm. *Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.



Standard Curve: This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.

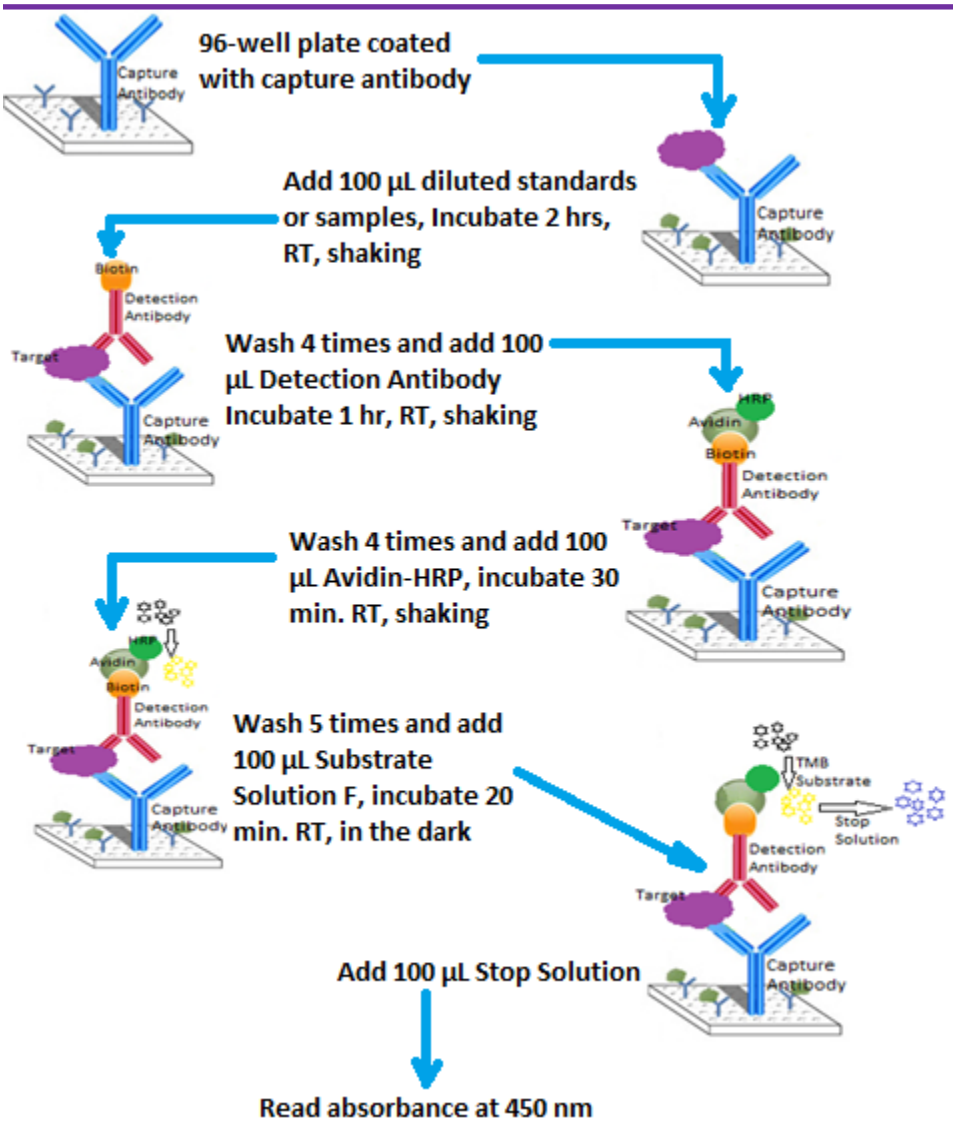
Data Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level PIIINP were tested 20 times on one plate, respectively.
Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level PIIINP were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/meanX100
Intra-Assay: CV<10%
Inter-Assay: CV<10%

Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. Substrate Solution F is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Summary of Assay Procedure



Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.

• Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

• Standard was reconstituted with less volume than required.
• One or more reagent incubation steps were too long.
• Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

• Samples contain no or below detectable levels of analyte.
• Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

• Pipetting errors may have occurred.
• Plate washing was inadequate or nonuniform.
• Samples were not homogenous.
• Samples or standard wells were contaminated.