IMMUNOPORT

SKU IP408987

TERIPARATIDE (TPT) CLIA KIT

Research Use Only
For *in vitro* applications - not for consumption

+1-(647)-3722995
INFO@IMMUNOPORT.COM

VERSION 1.0

INTENDED USE

To detect and quantitate Teriparatide (TPT) in Biological Solutions or Biological Fluids.

KIT CONTENTS & STORAGE

Strip Plate [96 wells]
Standard [2 vials]
Standard Diluent [20ml]
Detection Reagent A and B [120ul each]
Assay Diluent A and B [12ml each]
30X Wash solution [20ml]
Substrate A [10ml]
Substrate B [2ml]
Plate Sealing Membrane [4pcs]
User manual [1pc]
Zipper bag [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C for unused kit while the others should be at 4°C.

NOT INCLUDED

Distilled Water | Pipettes and Tips | Luminometer with lag time 30.0secs; read time 1.0 sec/well | PBS

SUMMARY

The microplate provided in this kit has been pre-coated with an antibody specific to teriparatide. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to teriparatide. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then the mixture of substrate A and B is added to generate glow light emission kinetics. Upon plate development, the intensity of the emitted light is proportional to the teriparatide level in the sample or standard.

CHARACTERISTICS

Sample Type: Biological Fluids / Solutions.
Detection Range: 5.49-4,000pg/mL
Minimum Detection Dose: 1.91pg/mL
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%
No Cross Reactivity with Analogues observed
Validity: 1 year from the date on manufacture
Storage: 2-8°C

Once Opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8 °C.

SAMPLE COLLECTION

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS on ice. The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter. The homogenates are centrifugated for 5 minutes at 5000×g to collect supernatant. Supernatant should subsequently be again centrifuges for 20 minutes at 1000Xg and used after removing any particulates. Please note that tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected CLIA results due to the impacts of certain chemicals

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

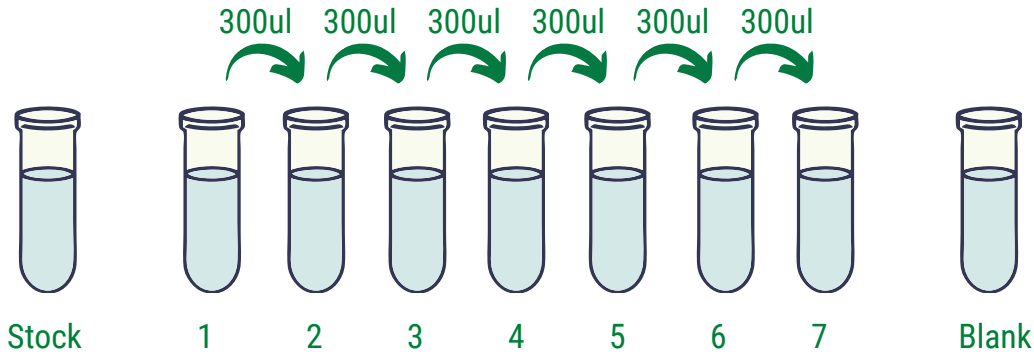
Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 20 minutes at 1000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles. Note: Samples should be centrifuged adequately and no hemolysis or granule was allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. **Standard** - Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently (do not foam). The concentration of the standard in the stock solution is 4000pg/mL. Please firstly transfer the stock solution to tube 1 and this serves as the highest standard. Then prepare 6 more tubes containing 0.6mL Standard Diluent and use the Tube 1 standard to produce the triple dilution series as shown below. Mix each tube thoroughly before the next transfer. Thus, set up 7 points of diluted standards: 4000, 1333.33, 444.44, 148.15, 49.38, 16.46, 5.49pg/mL and keep one more tube with only Standard Diluent to be used as blank (0ng/mL).



3. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.

4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **Working substrate solution** - Mix the substrate A and B by the ratio of 99:1 to make the substrate working solution. Mix thoroughly. For example, prepare 1,000μL Substrate working Solution with 990μL Substrate A + 10μL Substrate B.

PROCEDURE

1. Determine wells for diluted standard, blank and sample. Prepare at least seven wells for standard, 1 well for blank. Add 100μL each of prepared standards (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.

2. Remove the liquid of each well, don't wash.

3. Add 100μL of Detection Reagent A working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37°C.

4. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting or Invert the plate against absorbent paper.

5. Add 100μL of Detection Reagent B working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37°C.

6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.

7. Add 100μL of working Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 mins (can be increased to 20 mins) at 37°C . Protect from light.

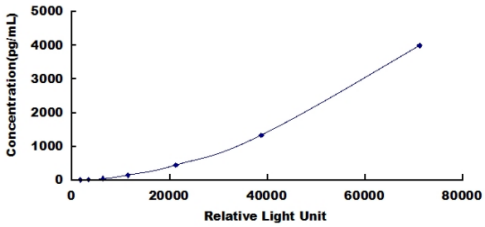
8. Measure the chemiluminescence signal in a microplate luminometer or as appropriate for the instrument used.

9. Relative light units (RLUs) may differ from different luminometers.The Immunoassay was optimized using a Hamamatsu luminometer. Other instruments may require settings to be adjusted.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard relative light unit (RLU). Create a standard curve on log-log graph paper, with teriparatide concentration on the y-axis and the RLU value on the x-axis. Draw the best fit straight line through the standard points and it can be determined by regression analysis. Using some plot software, such as curve expert 1.30, is also recommended. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

In order to make the calculation easier, we plot the RLU value of the standard (X-axis) against the known concentration of the standard (Y-axis), although concentration is indeed the independent variable while RLU value is the dependent variable. Further, in this figure below, in order to help the customer understand the assay visually, we provide the raw data (not the log of data). However, plotting log of the data to construct the curve is recommended. The RLU values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). This curve is provided for demonstration only.



TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets. We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

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