



User's Manual

Human Pleiotrophin ELISA Kit



DEIASL544



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Human Pleiotrophin ELISA kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human Pleiotrophin in serum, plasma and cell culture supernatants.

Principles of Testing

This assay employs an antibody specific for human Pleiotrophin coated on a 96-well plate. Standards and samples are pipetted into the wells and Pleiotrophin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human Pleiotrophin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Pleiotrophin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents And Materials Provided

1. Pleiotrophin Microplate (Item A): 96 wells (12 strips x 8 wells) coated with antiHuman Pleiotrophin. 1 month at 4°C*.
2. Wash Buffer Concentrate (20X) (Item B): 25 ml of 20X concentrated solution. 1 month at 4°C.
3. Standard Protein (Item C): 2 vials of Human Pleiotrophin. 1 vial is enough to run each standard in duplicate. 1 week at -80°C.
4. Detection Antibody Pleiotrophin (Item F): 2 vials of biotinylated anti-Human Pleiotrophin. Each vial is enough to assay half the microplate. 5 days at 4°C.
5. HRP-Streptavidin Concentrate (Item G): 200 µl 700X concentrated HRP-conjugated streptavidin. Do not store and reuse.
6. TMB One-Step Substrate Reagent (Item H): 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution.
7. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
8. Assay Diluent C (Item L): 30 ml of diluent buffer.
9. Assay Diluent B (Item E): 15 ml of 5X concentrated buffer. 1 month at 4°C.

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.

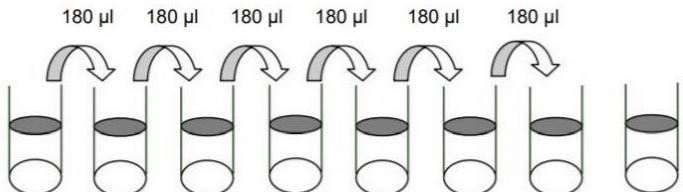
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C.

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water before use.
3. Sample dilution: Assay Diluent C (Item L) should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 2 fold. Note: Levels of Pleiotrophin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of standard: Briefly spin a vial of Item C. Add 400 µl Assay Diluent C (Item L) into Item C vial to prepare a 300 ng/ml standard solution. Dissolve the powder thoroughly by a gentle mix. Pipette 270 µl Assay Diluent C into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Assay Diluent C serves as the zero standard (0 ng/ml).



	Std1	Std2	Std3	Std4	Std5	Std6	Std7	Zero Standard
Diluent volume	Item C + 400 µl	270 µl	270 µl	270 µl	270 µl	270 µl	270 µl	270 µl
Conc.	300 ng/ml	120 ng/ml	48 ng/ml	19.20 ng/ml	7.680 ng/ml	3.072 ng/ml	1.229 ng/ml	0 ng/ml

5. If the Wash Concentrate (20X) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1X Assay Diluent B (Item E) into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B (Item E) and used in step 5 of Part VI Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted 700-fold with 1X Assay Diluent B (Item E). For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 14 ml 1X Assay Diluent B to prepare a 700-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix Well.

Assay Procedure

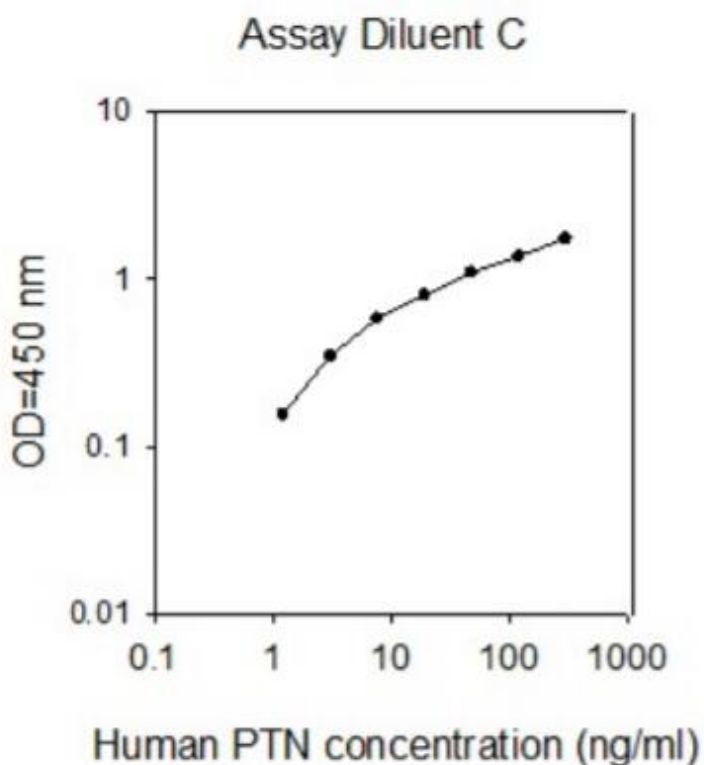
1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.
4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. 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.
5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
6. Discard the solution. Repeat the wash as in step 4.
7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
8. Discard the solution. Repeat the wash as in step 4.
9. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
10. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.



Detection Range

1.3 ng/ml - 300 ng/ml

Sensitivity

The minimum detectable dose of Human Pleiotrophin was determined to be 1.3 ng/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Specificity

This ELISA antibody pair detects human Pleiotrophin. Other species not determined.

Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected Range (%)	93.77 78-110	116.0 98-134	98.21 91-105
1:4	Average % of Expected Range (%)	128.9 110-148	138.8 131-146	132.8 127-139

Recovery

Recovery was determined by spiking various levels of Human Pleiotrophin into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	111.4	72-144
Plasma	115.1	90-143
Cell culture media	125.4	101-148

Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%