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（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Mouse PDGFRL (Platelet Derived Growth Factor Receptor Like Protein) CLIA Kit

Catalog No: MBS2535493

96T

This manual must be read attentively and completely before using this product.

Intended use

This CLIA kit applies to the in vitro quantitative determination of PDGFRL concentrations in serum, plasma and other biological fluids.

Sensitivity

The minimum detectable dose of PDGFRL is 9.375pg/mL (The sensitivity of this assay, or lowest detectable limit (LDL) was defined as the lowest protein concentration that could be differentiated from zero).

Detection Range

15.625-1000pg/mL

Specificity

This kit recognizes natural and recombinant PDGFRL. No significant cross-reactivity or interference between PDGFRL and analogues was observed.

Note：

Limited by existing techniques, cross reaction may still exist, as it is impossible for us to complete the cross-reactivity detection between PDGFRL and all the analogues.

Repeatability

Coefficient of variation were<15%.

Statement: Thank you for choosing our products. This product is produced by using raw material from world-renowned manufacturer and professional manufacturing technology of CLIA kits. Please read the instructions carefully before use and check all the reagent compositions!

Storage: All the reagents in the kit should be stored according to the labels on vials. Substrate Reagent shouldn't be kept at -20℃ (Check!). Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination. If not to store reagents according to above suggestions, erroneous results may occur.

Kit Components：

Item	Specifications	Storage
Micro CLIA Plate	8 ×12 wells	4℃/-20℃#
Reference Standard	2 vials	4℃/-20℃#
Reference Standard & Sample Diluent	1vial 20mL	4℃
Concentrated Biotinylated Detection Ab	1vial 120μL	4℃/-20℃#
Biotinylated Detection Ab Diluent	1vial 10mL	4℃
Concentrated HRP Conjugate	1vial 120μL	4℃(shading light)
HRP Conjugate Diluent	1vial 10mL	4℃
Concentrated Wash Buffer (25×)	1vial 30mL	4℃
Substrate Reagent A	1vial 5mL	4℃(shading light)
Substrate Reagent B	1vial 5mL	4℃(shading light)
Plate Sealer	5pieces	
Manual	1 copy	
Desiccant	1 pack	
Certificate of Analysis	1 copy	

#: keep the kit at 4℃ if it's used within 30 days, keep at -20℃ for longer storage.

Test principle

This kit uses Sandwich-CLIA as the method. The micro CLIA plate provided in this kit has been pre-coated with an antibody specific to PDGFRL. Standards or samples are added to the appropriate micro CLIA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for PDGFRL and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain PDGFRL, biotinylated detection antibody and Avidin-HRP conjugate will appear fluorescence. The Relative light unit (RLU) value is measured spectrophotometrically by the Chemiluminescence immunoassay analyzer. The RLU value is positively associated with the concentration of PDGFRL. You can calculate the concentration of PDGFRL in the samples by comparing the RLU value of the samples to the standard curve.

Sample collection and storage

Samples should be clear and transparent and be centrifuged to remove suspended solids.

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4℃ before centrifugation for 15 minutes at 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8℃ within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Hemolysis samples are not suitable for CLIA assay!

Cell culture supernate: Centrifuge supernate for 20 minutes to remove insoluble impurity and cell debris at 1000×g at 2 - 8°C. Collect the clear supernate and carry out the assay immediately.

Tissue homogenates: You'd better get detailed references from other literatures before assay aiming at different tissue types. For general information, hemolysis blood may affect the result, so you should mince the tissues to small pieces and rinse them in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (the volume depends on the weight of the tissue) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5minutes at 5000×g to get the supernate.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2-8℃. Collect the supernatant and carry out the assay immediately.

Note:

1. Samples should be used within 7 days when stored at 2-8℃, otherwise samples must be divided and stored at -20℃ (≤1month) or -80℃ (≤6months) to avoid the loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles.
2. Please take the samples to room temperature (18-25℃) without extra heating before performing the assay.
3. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample preparation

1. We are only responsible for the kit itself, but not for the samples consumed during the experiment. The user should calculate the possible amount of the samples needed in the whole test. Reserving sufficient samples in advance is recommended.
2. If the samples are not mentioned in this manual, a pre-experiment to determine the validity of the kit is necessary.

3. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected CLIA results due to the impacts of certain chemicals.
4. Due to the possibility of mismatching between antigen from other origins and antibodies used in our kits, some native or recombinant proteins from other manufacturers may not be detected by our kits.
5. Influenced by factors including cell viability, cell number or sampling time, molecular from cells culture supernatant may not be detected by the kit.
6. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Other supplies required

Chemiluminescence immunoassay analyzer
High-precision transferpettor, EP tubes and disposable pipette tips
37℃ Incubator
Deionized or distilled water
Absorbent paper
Loading slot for Wash Buffer

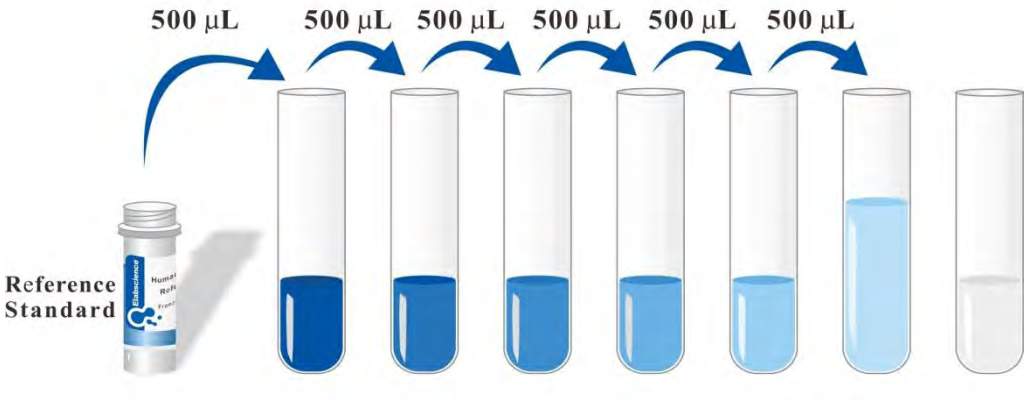
Reagent preparation

Bring all reagents to room temperature (18-25℃) before use.

Wash Buffer - Dilute 30 mL of Concentrated Wash Buffer into 750 mL of Wash Buffer with deionized or distilled water. Put unused solution back at 4℃. If crystals have formed in the concentrate, you can warm it with 40℃ water bath (Heating temperature should not exceed 50℃) and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.

Standard – Prepare standard within 15 minutes before use. Centrifuge at 10,000×g for 1 minute, and reconstitute the Standard with **1.0mL** of Reference Standard &Sample Diluent. Tighten the lid, let it stand for 10 minutes and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 1000pg/mL. Then make serial dilutions as needed (making serial dilution in the wells directly is not permitted). The recommended concentrations are as follows:**1000、500、250、125、62.5、31.25、15.625、0 pg/mL**. If you want to make standard solution at the concentration of 500pg/mL, you should take0.5mL standard at 1000pg/mL, add it to an EP tube with 0.5mL Reference Standard&Sample diluent, and mix it.Procedures to prepare the remained concentrations are all the same. The undiluted standard serves as the highest standard (1000pg/mL). The Reference Standard&Sample Diluent serves as the zero (0 pg/mL).

(Standards can also be diluted according to the actual amount，such as 200μL/tube)



1	2	3	4	5	6	7	8	tube
1000	500	250	125	62.5	31.25	15.625	0	pg/mL

Biotinylated Detection Ab – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Centrifuge the stock tube before use, dilute the concentrated Biotinylated Detection Ab to the working concentration using Biotinylated Detection Ab Diluent (1:100).

Concentrated HRP Conjugate – Calculate the required amount before experiment (100µL/well). In actual preparation, you should prepare 100~200µL more. Dilute the Concentrated HRP Conjugate to the working concentration using Concentrated HRP Conjugate Diluent (1:100).

Substrate Reagent: As it is sensitive to light and contaminants, so you shouldn't open the vial until you need it! The needed dosage of the reagent can be aspirated with sterilized tips and the unused residual reagent shouldn't be dumped back into the vial again. Please mix the Substrate Reagent A and B with equal volumes before use!

Note: Please don't prepare the reagent directly in the Diluent vials provided in the kit. Contaminated water or container for reagent preparation will influence the result.

Washing Procedure:

1. **Automated Washer:** Add 350µL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** Add 350µLWash Buffer into each well, soak it for 1~2minutes. After the last wash, decant any remaining Wash Buffer by inverting the plate and blotting it dry by rapping it firmly against clean and toweling absorbent paper on a hard surface.

Assay procedure

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** It's recommended that all samples and standards be assayed in duplicate.

1. **Add Sample:** Add 100μL of Standard, Blank, or Sample per well. The blank well is added with Reference Standard & Sample Diluent. Solutions are added to the bottom of micro CLIA plate well, avoid inside wall touching and foaming as possible. Mix it gently. Cover the plate with sealer we provided. Incubate for 90 minutes at 37℃.
2. **Biotinylated Detection Ab:** Remove the liquid of each well, don't wash. Immediately add 100μL of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer. Gently tap the plate to ensure thorough mixing. Incubate for 1 hour at 37°C.
3. **Wash:** Aspirate each well and wash, repeating the process three times. Wash by filling each well with Wash Buffer (approximately 350μL) (a squirt bottle, multi-channel pipette, manifold dispenser or automated washer are needed). Complete removal of liquid at each step is essential. After the last wash, remove remained Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.
4. **HRP Conjugate:** Add 100μL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.
5. **Wash:** Repeat the wash process for five times as conducted in step 3.
6. **Substrate:** Add 100μL of Substrate Mixture Solution to each well. Cover with a new Plate sealer. Incubate for not more than 5 minutes at 37°C. Protect the plate from light.
7. **RLU Value Measurement:** Determine the RLU value of each well at once after the substrate reaction time. You should open the Chemiluminescence immunoassay analyzer ahead, preheat the instrument, and set the testing parameters.
8. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

Important Note:

1. **CLIA Plate**: The just opened CLIA Plate may appear water-like substance, which is normal and will not have any impact on the experimental results.
2. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. Parallel measurement is recommended.

3. **Incubation**: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Do not let the wells dry at any time during the assay. Strict compliance with the given incubation time and temperature.
4. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the micro-titer plate reader.
5. **Reagent Preparation**: As the volume of Concentrated Biotinylated Detection Ab and Concentrated HRP Conjugate is very small, liquid may adhere to the tube wall or tube cap when being transported. You better hand-throw it or centrifugal it for 1 minute at 1000rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, working solutions of Detection Ab and HRP Conjugate according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10μL for once pipetting. Do not reuse standard solution, working solution of Detection Ab and HRP Conjugate, which have been diluted. If you need to use standard repeatedly, you can divide the standard into a small pack according to the amount of each assay, keep them at -20～-80℃ and avoid repeated freezing and thawing.
6. **Reaction Time Control:** Please control reaction time strictly following this product description!
7. **Substrate:** Substrate Solution is easily contaminated. Please protect it from light.
8. **Mixing:** You'd better use micro-oscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no micro-oscillator available, you can knock the CLIA plate frame gently with your finger before reaction.
9. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
10. Do not use components from different batches of kit(washing buffer can be an exception)
11. To avoid cross-contamination, change pipette tips between adding of each standard level, between sample adding, and between reagent adding. Also, use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of results

Average the duplicate readings for each standard and samples and subtract the average zero standard RLU value. Create a standard curve by plotting the mean RLU value for each standard on the y-axis or x-axis against the concentration on the x-axis or y-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using RLU values and concentrations of standard sample. The software will calculate the concentration of samples after entering the RLU value of samples. Also, you can enter the corresponding fitting equation and RLU value of samples into Excel to get the concentration of samples. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the RLU value of the sample surpasses the upper limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

Typical data

As the RLU value of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish standard curve for each test. Typical standard curve and data below is provided for reference only.

SUMMARY

1. Add 100μL standard or sample to each well. Incubate 90mintues at 37℃
2. Remove the liquid. Add 100μLBiotinylated Detection Ab. Incubate 1 hour at 37℃
3. Aspirate and wash 3 times
4. Add 100μL HRP Conjugate. Incubate 30 minutes at 37℃
5. Aspirate and wash 5 times
6. Add 100μL Substrate Mixture Solution. Incubate 5 minutes at 37℃
7. Read immediately after the substrate reaction time
8. Calculation of results

Troubleshooting

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure briefly spin the vial of standard and dissolve the powder thoroughly by a gentle mix.
	Wells not completely aspirated	Completely aspirate wells between steps.
Low fluorescence	Too long incubation times	Ensure precise incubation time
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes	Check pipettes and ensure correct preparation
	Improper dilution	
Large CV	Inaccurate pipetting	Check pipettes
High background	Concentration of detector too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the CLIA kit	All the reagents should be stored according to the instructions
	Too long incubation time	Ensure precise incubation time

Declaration:

1. Limited by current conditions and scientific technology, we can't completely conduct the comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. The final experimental results will be closely related to the validity of products, operation skills of the operators and the experimental environments. Please make sure that sufficient samples are available.
3. To get the best results, please only use the reagents supplied by the manufacturer and strictly comply with the instructions in the description!
4. Incorrect results may led by wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for Micro-plate reader. Please read the instruction carefully and adjust the instrument prior to the experiment.
5. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled.
6. Every kit has strictly passed QC test. However, results from end users might be inconsistent with our data due to some unexpected reasons such as transportation conditions, different lab equipments, and so on. Intra-assay variance among kits from different batches might arise from above reasons, too.
7. Valid period: 6 months.