(For scientific research use only, not for clinical diagnosis!)

Human D-dimer (D2D) Quantitative Detection Kit (ELISA) Instructions for Use Specifications: 48T/96T

Please read the instructions carefully before use. If you have any questions, please contact us through the following methods: Official hotline: 400-999-8863 Technical phone: 18358180525 Email: UpingBio@163.com Company website: www.upingbio.com For the specific shelf life, please see the outer packaging label of the kit. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we can serve you more efficiently.

Kit performance Physical properties: Each liquid component is clear and transparent, with no precipitation or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Standard curve linearity: Calibrator dose-response curve correlation coefficient r value, greater than or equal to 0.9900.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Sensitivity: The lowest detectable dose is less than 1.0 ng/mL.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes native and recombinant human D-dimers (D2D) without crossover to structural analogs.

Stability: Stored at 2℃-8℃, validity period is 6 months.

Detection range: 25-400 ng/mL.

Purpose: Used to detect the concentration of human D-dimer (D2D) in serum, plasma, cell culture supernatant and other samples.

Experimental principle The kit uses enzyme-linked immunoassay method. Biotin-labeled D2D is used, and the purified anti-D2D antibody is coated on the microwell plate. In the competitive inhibition reaction, a certain amount of solid-phase antibody is used to inhibit the competitive reaction with biotin-labeled D2D and non-labeled antigen (calibrator or specimen). The antibody The amount of binding to biotin-labeled D2D is inhibited by the amount of non-labeled antigen. The greater the amount of non-labeled antigen, the less the antibody will bind to biotin-labeled D2D, and vice versa. After the reaction is balanced, a solid-phase antibody-biotinylation is formed. D2D, and then add enzyme-labeled avidin to form a solid-phase antibody-biotinylated

D2D-enzyme-labeled-avidin complex. After adding substrate for color development, use a microplate reader to measure the absorbance (OD value) at a wavelength of 450 nm. As the D2D concentration increases, the OD value gradually decreases with a good linear relationship. This kit has the characteristics of high sensitivity, strong specificity, good repeatability, simple and rapid operation, and has reliable detection performance for the reduction or increase of D2D in serum.

Kit components and storage: Store unopened kits at 2-8

degrees Celsius. Do not use expired kits.

Components	quantity	Main ingredients
Calibrator	0.5ml/tube*6 tubes	6 concentration standards formulated for antigen
coated microplate	96T/48T	Pre-coated solid phase antibodies
HRP labeled antibodies	6mL	HRP-labeled detection antibodies
biotinylated antigen	6mL	Detect antigen
sample diluent	6mL	Phosphate buffer
Substrate solution A	6mL	hydrogen peroxide working fluid
Substrate solution B	6mL	TMB working fluid
stop solution	6mL	acidic solution
20×concentrated washing liquid	30mL	PBS with 0.15% Tween20
manual	1 serving	--
ziplock bag	1	--
Self-adhesive	2 tablets	--

The concentrations of calibrators are: 400, 200, 100, 50, 25, 0 ng/mL.

Note: 1: Before use, please check whether the label and quantity of

the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have

not been contaminated since the last use. 3: If the enzyme plate is not used up in a

single time, remember to seal it and store it at 2-8°C.

Self-prepared test equipment required for the test

(not provided, but can be purchased with

assistance) 1. Standard specification microplate

reader.

2. Automatic plate washing machine.

3. Oscillator.

4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

Restrictions on the kit: 1. For scientific

research use only and not for clinical

diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.

3. Do not mix with kits or components from other manufacturers.

4. Use the sample diluent provided with the kit.

5. If the sample value is higher than the highest standard concentration value, please dilute

the sample appropriately and then re-measure. 6. Human anti-mouse and other heterophilic

antibodies present in the sample to be tested will interfere with the test results. Please

eliminate this factor before testing.

7. The test results obtained by other methods are not directly comparable to the test results of this kit.

Notes: 1) This kit is for in vitro research only and

not for clinical diagnosis.

2) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

3) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

4) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not allow the microwells to dry out during incubation.

5) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.

6) The substrate chromogenic solution should be colorless or very light in color.

7) Avoid cross-contamination of reagents and specimens to avoid erroneous results.

8) Avoid direct exposure to strong light during storage and incubation.

9) Equilibrate to room temperature before opening the sealed bag to prevent water droplets from condensing on the cold slats.

10) Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.

11) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of 450±10nm, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.

12) Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.

13) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.

14) Do not use expired reagents.

Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 120 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

3. Substrate: Substrate solutions A and B, mix thoroughly at a volume of 1:1 before use, and use within 15 minutes after mixing.

Operation procedure 1. Move various reagents to room temperature and equilibrate for half an hour. Take the concentrated washing solution, dilute it with distilled water 1:20 according to the number of tests in the current batch, mix well and set aside.

2. Take out the pre-coated plate from the sealed bag, set a blank control well without adding any liquid; set 2 holes for each calibrator, add 50 μl of the corresponding calibrator to each well; add the serum to be tested directly to each of the remaining detection holes. Or 50μl of quality control product.

3. Add 50 μl of biotinylated antigen to all wells except the blank well, mix well, attach sealing film, and incubate at 37℃ for 60 minutes.

4. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

5. Add 50 μl of enzyme-labeled avidin to each well (except the blank control well), mix well, attach a sealing film, and incubate at 37℃ for 30 minutes.

6. Manual plate washing: discard the liquid in the wells, fill each well with washing solution, let stand for 10 seconds and spin dry, repeat 3 times and pat dry. Wash the plate with a plate washer: select the washing program 3 times and pat dry after washing the plate.

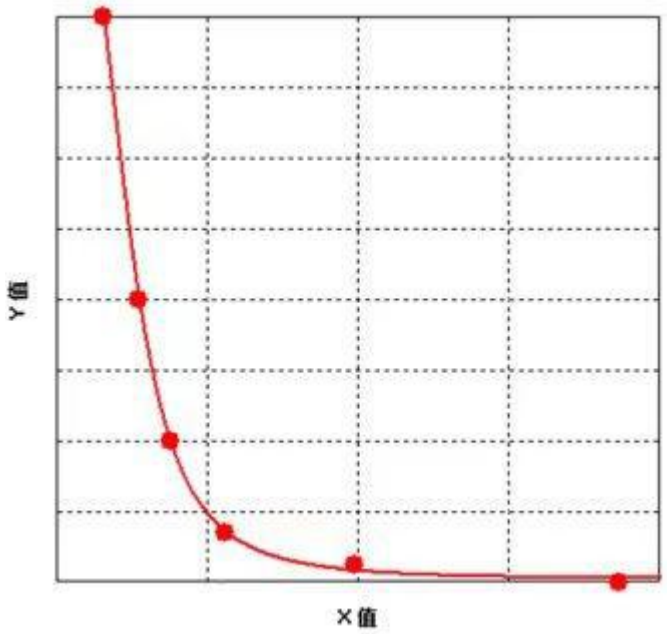
7. Add 50 μl of chromogen A and 50 μl of chromogen B to each well. After shaking and mixing, place at 37℃ to develop color in the dark for 15 minutes. Add 50 μl of stop solution to each well.

8. Use a microplate reader to read, take the wavelength of 450nm, first use the blank control well to adjust the zero point, and then measure the optical density value (OD value) of each well.

Result calculation

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]

10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.



(Schematic diagram, for reference only)

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used laths and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]

Problem Description	Possible Causes	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	The experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
	Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to