Technical Manual

QuickStep Human D2D (D-Dimer) ELISA Kit

- **Catalogue Code: QSES024**
- **Sandwich ELISA Kit**
- **Research Use Only**

1. Description and Principle

QuickStep is a revolutionary innovation in pre-coated ELISA kit technologies. Using highly validated antigens & antibodies coupled with optimized buffer systems, QuickStep guarantees super-sensitive quantitative results in 90 minutes with a 1-wash protocol.

How Do QuickStep ELISA Kits Work in 90 minutes?

This Quickstep kit uses a Sandwich ELISA technique to quantify D2D concentrations in Serum and plasma samples.

The QuickStep ELISA plate provided in this kit is pre-coated with an antibody specific to Human D2D (D-Dimer). Samples (or Standards) and Horseradish Peroxidase (HRP) linked antibody specific for D2D are added to the micro ELISA plate wells. D2D in samples (or standards) combines with the coated antibody and HRP linked detection antibody specific to D2D. Excess conjugate and unbound sample or standard are washed from the plate and a TMB substrate solution is the added to each well. The enzyme-substrate reaction is terminated by the addition of a stop solution and the color change is measured with a microplate reader at a wavelength of 450 nm ± 2 nm. The concentration of D2D in the samples is determined by comparing the OD of the samples to the standard curve.

2. Key features and Sample Types

Sensitivity: 53.10 pg/mL

Detection Range: 125.00-4000 pg/mL

Repeatability: Coefficient of variation (CV) is < 10%

ELISA Type: Sandwich

Specificity: This kit recognizes D2D in Human samples. No significant cross reactivity or interference between Human D2D (D-Dimer) and analogues was observed.

SUMMARY

1. Add 50µL standard or sample to each well. Immediately add 50µL HRP linked Ab working solution to each well. Incubate for 60 min at 37°C.
2. Aspirate and wash 5 times.
3. Add 90µL Substrate Reagent. Incubate for 15 min at 37°C.
4. Add 50µL Stop Solution.
5. Read at 450 nm immediately.
6. Calculation of results

3. Kit Contents

Product	Size	Cat. Code
Human D2D (D-Dimer) ELISA Kit	48/96 assays	QSES024

Each kit contains reagents for 48/96 assays in a 48/96 well plate including:

Item	Specifications	Storage
Micro ELISA Plate(Dismountable)	8 wells ×12 strips	2-8°C, 1 months
Reference Standard	2 vials	2-8°C, use the reconstituted standard within 24h
Concentrated HRP Linked Detection Ab(100×)	1 vial, 60 µL	2-8°C(Protect from light)
Reference Standard & Sample Diluent	1 vial, 20 mL	2-8°C
HRP Linked Ab Diluent	1 vial, 14 mL	2-8°C
Concentrated Wash Buffer(25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	2-8°C(Protect from light)
Stop Solution	1 vial, 10 mL	2-8°C
Plate Sealer	5 pieces	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial contamination. The volume of reagents in the actual kit is in excess of the volumes marked on the labels.

Additional Materials required

1. Microplate reader with 450 nm wavelength filter
2. High-precision transfer pipette, EP tubes and disposable pipette tips
3. Incubator capable of maintaining 37°C
4. Deionized or distilled water
5. Absorbent paper
6. Loading slot for Wash Buffer

4. Shipping and Storage

The unopened kit can be stored at 2-8°C for six months. After opening the kit, the unused wells and reagents should be stored according to the vial/bottle labels.

5. Sample Preparation

Serum: Allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 min at 1000×g at 2~8°C. Collect the supernatant to carry out the assay. Blood collection tubes should be disposable and be endotoxin-free.

Plasma: Collect plasma using EDTA-Na^2 as an anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8°C within 30 min of collection. Collect the supernatant to carry out the assay. Hemolysed samples are not suitable for ELISA assay.

Cell lysates: For adherent cells, gently wash the cells with pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension in a tube and centrifuge for 5 min at 1000×g. Discard the medium and wash the cells 3 times with precooled PBS. For each 1×106 cells, add 150-250µL of pre-cooled PBS to keep the cells suspended. Optimal cell concentration is 1 million/ml. To release cellular components, dilute the cell pellet in PBS and use 3-4 freeze thaw cycles in liquid Nitrogen (commercial lyses buffers can be used according to manufacturer's instructions). Centrifuge at 4°C for 20 mins at 2000-3000 rpm to pellet debris and remove clear supernatant to clean microcentrifuge tube for ELISA or storage.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 min at 5000×g to get the supernatant.

Cell culture supernatant: Centrifuge samples for 20 min at 1000×g at 2~ 8°C. Collect the supernatant to carry out the assay

Saliva: Remove particulates by centrifugation for 10 minutes at 4000×g at 2-8°C. Collect the supernatant to carry out the assay. Fresh saliva samples are recommended.

Urine: Use a sterile container to collect urine samples. Remove particulates by centrifugation for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to carry out the assay.

Stool: Collect feces, suspend the stool with PBS (0.01M, pH=7.4), oscillate on the ice for 15 minutes and then centrifuge for 5 minutes at 5000×g at 2- 8°C. Collect the supernatant to carry out the assay.

Other biological fluids: Please contact tech-support for details.

Note

1. Samples should be assayed within 7 days when stored at 2-8°C. Otherwise samples must be aliquoted and stored at -20°C (≤1 month) or -80°C (≤3 months). Avoid repeated freeze-thaw cycles.
2. Samples with high hemolysis or lipids are not suitable for this assay.
3. Determine the protein 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.

Dilution Method

It is recommended to dilute normal plasma samples at 20-50 fold.

Predict the concentration range of the sample in advance. If your test sample needs to be diluted, refer to the dilution method as follows:

- **100 fold dilution:** One-step dilution. Add 5 µL sample to 495 µL sample diluent to yield 100 fold dilution.
- **1,000 fold dilution:** Two-step dilution. Add 5 µL sample to 95 µL sample diluent to yield 20 fold dilution. Then add 5 µL of the 20 fold diluted sample to 245 µL sample diluent. This 2-step process gives a 1,000 fold diluted sample.
- **100,000 fold dilution:** Three-step dilution. Add 5 µL sample to 195 µL sample diluent to yield 40 fold dilution. Then add 5 µL of the 40 fold diluted sample to 245 µL sample diluent to yield a 2,000 fold dilution. Finally, add 5 µL of the 2,000 fold diluted sample to 245 µL sample diluent. This 3-step process gives a 100,000 fold diluted sample.

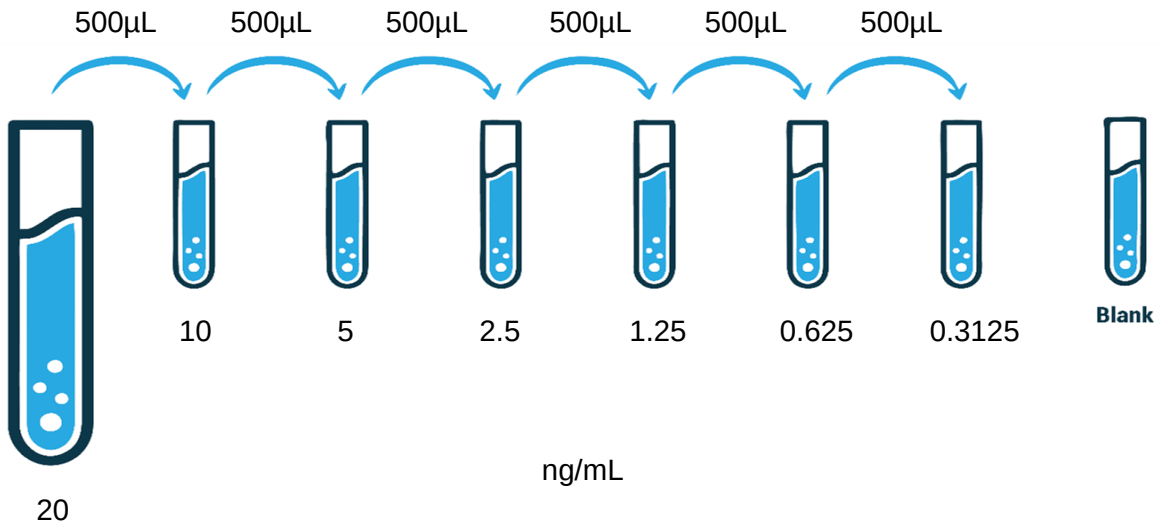
6. Protocol

1. Bring all reagents to room temperature (18~25°C) before use. Follow the microplate reader manual for set-up and preheat it for 15 min before OD measurement. The unused wells and reagents should be stored according to the vial/bottle labels.

2. Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer. Note: if crystals have formed in the concentrate, warm it in a 40°C water bath and mix it gently until the crystals have completely dissolved.

3. Standard working solution: Centrifuge the standard at 10,000×g for 1 min. Add 1 mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a working solution of 20 ng/mL (or add 1 mL of Reference Standard and Sample Diluent, let it stand for 1-2 min and then vortex thoroughly at low speed. Bubbles generated during vortexing can be removed by centrifuging at low speed). Then make serial dilutions as needed. The recommended dilution gradient is as follows: 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0 ng/mL. Note: the last tube is regarded as a blank. Don’t pipette solution into it from the former tube.

Dilution method: Take 7 EP tubes, add 500µL of Reference Standard & Sample Diluent to each tube. Pipette 500µL of the 20 ng/mL working solution to the first tube and mix up to produce a 10 ng/mL working solution. Pipette 500µL of the solution from the former tube into the latter one according to these steps. The illustration below is for reference.



5. HRP linked Ab working solution: Calculate the required amount before the experiment (50μL/well). In preparation, slightly more than calculated should be prepared. Centrifuge the Concentrated HRP linked Ab at 800×g for 1 min. Dilute the 100× Concentrated HRP linked Ab to 1× working solution with HRP linked Ab Diluent (1:99).

7. Assay procedure

1. Add standard, test sample and control (50 uL) wells to the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate. Note: add all solutions to the bottom of the plate wells while avoiding contact with the well walls. Ensure solutions do not foam when adding to the wells.

2. Immediately add 50μL of HRP linked Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 60 min at 37°C

3. Aspirate or decant the solution from the plate and add 350μL of wash buffer to each well and incubate for 1-2 minutes at room temperature. Aspirate the solution from each well and clap the plate on absorbent filter paper to dry. Repeat this process 5 times. Note: a microplate washer can be used in this step and other wash steps.

4. Add 90μL of Substrate Reagent to each well. Cover with a new plate seal and incubate for approximately 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not by more than 30min.

5. Add 50 μL of Stop Solution to each well. Note: Adding the stop solution should be done in the same order as the substrate solution.

6. Determine the optical density (OD value) of each well immediately with a microplate reader set at 450 nm.

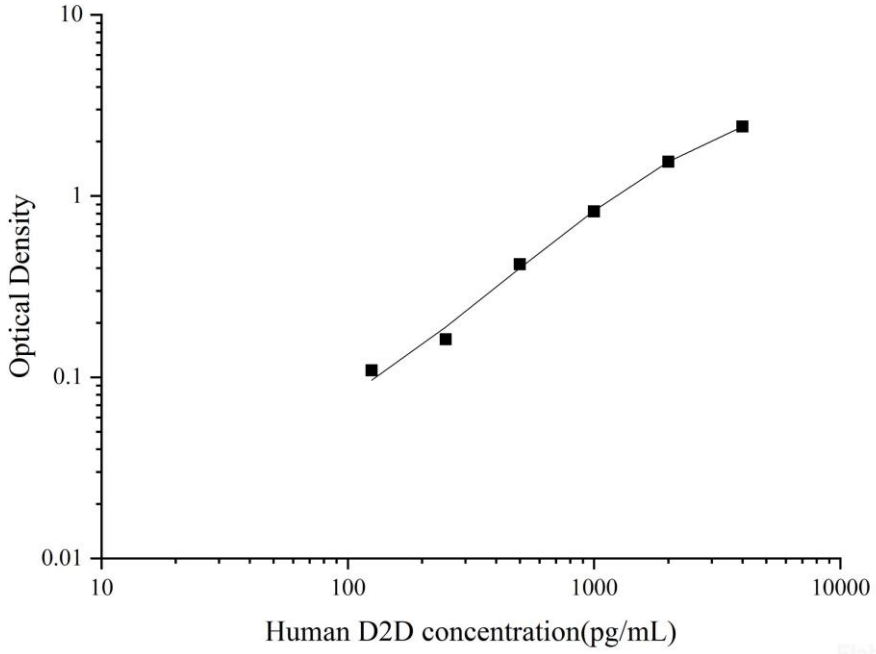
8. Data analysis

Average the duplicate readings for each standard and samples. Plot a four parameter logistic (4-PL) curve on log-log axis, with standard concentration on the x-axis and OD values on the y-axis.

If the OD of the sample under the lowest limit of the standard curve, you should re-test it with an appropriate dilution. The actual concentration is the calculated concentration multiplied by the dilution factor.

Typical data

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should generate a standard curve for each experiment. Typical standard curve is provided below (for reference only).



Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and high level Human D2D (D-Dimer) were tested 20 times on one plate.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	193.00	640.27	1476.29	177.05	587.91	1461.37
Standard deviation	11.04	32.91	53.74	8.89	23.69	47.64
CV (%)	5.72	5.14	3.64	5.02	4.03	3.26

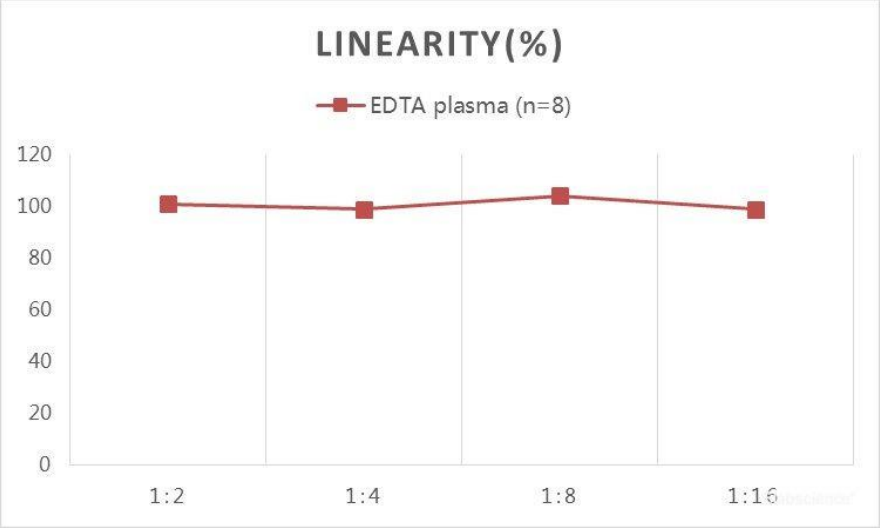
Recovery

The recovery of Human D2D (D-Dimer) spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Sample Type	Range (%)	Average Recovery (%)
Serum(n=8)	93-108	100
EDTA plasma(n=8)	90-104	96

Linearity

Samples were spiked with high concentrations of Human D2D (D-Dimer) and diluted with Reference Standard & Sample Diluent to produce samples with values within the range of the assay.



9. Important General Notes:

Problem	Causes	Solutions
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Centrifuge the standard vial and ensure contents are dissolved thoroughly.
	Wells are not completely aspirated	Completely aspirate wells in between steps.
Low signal	Insufficient incubation time	Lengthen/Shorten 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	
	HRP conjugate inactive or TMB failure	Mix HRP conjugate and TMB = rapid colour change.
Deep colour but low value	Plate reader setting is not optimal	Verify the wavelength and filter setting on the Microplate reader.
		Pre-heat Microplate Reader.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Concentration of target protein is too high	Use recommended dilution factor.
	Plate is insufficiently washed	Review the manual for proper washing procedure. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	All the reagents should be stored according to the instructions.
	Stop solution is not added	Stop solution should be added to each well before measurement.

Additional Notes:

1. Wear lab coats, eye goggles and latex gloves for protection. Perform the experiment following the national safety guidelines for biological laboratories, especially when using blood samples or other bodily fluids.

2. A freshly opened ELISA Plate may appear to have a water-like substance, this is normal and will not have any impact on the experimental results.

3. Do not reuse the reconstituted standard and HRP linked detection Ab working solution. The unused concentrated HRP linked detection Ab (100×) and other stock solutions should be stored according to the storage conditions in the above table.

4. The microplate reader should have a 450(±10 nm) filter installed and a detector that can detect this wavelength. The optical density should be within 0~3.5.

5. Do not mix or use components from other lots.

6. Change pipette tips in between adding standards, sample additions and reagent additions. Also, use separate reservoirs for each reagent.

7. The kit should not be used beyond the expiration date on the kit label.

Declaration

1. Limited by current scientific technology, we can't conduct comprehensive identification and analysis on all the raw materials provided. There might be some qualitative and technical risks for users using the kit.

2. This assay is designed to eliminate interference by factors present in biological samples. Until all factors have been tested in the ELISA immunoassay, the possibility of interference cannot be excluded.

3. The final experimental results will be closely related to the validity of products, operational skills and the experimental environment. Please make sure that sufficient samples are available.

3. To get the best results, only use the reagents supplied with this kit and strictly comply with the instructions.

4. Incorrect results may occur from incorrect reagent preparation and loading, as well as incorrect parameter settings of the Micro-plate reader. Please read the instructions carefully and adjust the instrument prior to the experiment.

5. Each kit passes a strict QC procedure. However, results from end users might be inconsistent with our data due to some variables such as transportation conditions, different lab equipment, and so on. Intra-assay variance among kits from different batches might also arise.

6. The kit is designed for research use only, we will not be responsible for any issues if the kit is applied in clinical diagnosis or any other related procedures.

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