D-Dimer ELISA Kit (Human)
(OKRC00358)
Lot# KF0560
Instructions for Use

For the quantitative measurement of D-Dimer in Human Cell Culture Supernatants | Plasma | Serum.

This product is intended for research use only.

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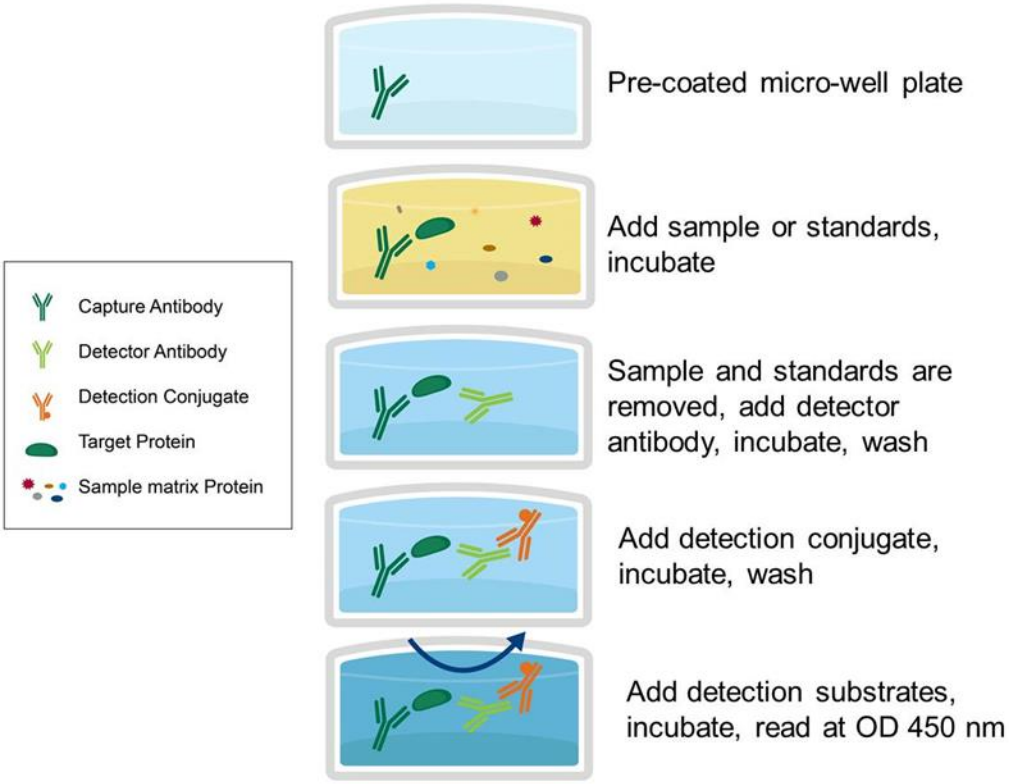
1. Background

Principle

The Aviva Systems Biology D-Dimer ELISA kit (Human) is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human D-Dimer in Cell Culture Supernatants | Plasma | Serum. This assay employs an antibody specific for Human D-Dimer coated on a 96-well plate. Standards and samples are pipetted into the wells and D-Dimer present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human D-Dimer 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 D-Dimer bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

General Specifications	
Specificity	Human D-Dimer Target Alias: D-Dimer
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

• Open kit immediately upon receipt. Store components at -20°C (NOTE: exceptions below) for long term storage or 4°C for short term use. Avoid any freeze/thaw cycles.

4. Kit Components

• The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
D-Dimer Microplate	96 Wells (12 x 8 Well strips)	-20°C for 1 year 4°C for 6 months
D-Dimer Lyophilized Standard	2 vials	
Biotinylated D-Dimer Detector Antibody	2 vials	
900X Streptavidin-HRP Conjugate	1 x 200 uL	
5X Assay Diluent	2 x 15 mL	
20X Wash Buffer	1 x 25 mL	
Stop Solution	1 x 8 mL	
TMB Substrate	1 x 12 mL	

5. Precautions

• Read instructions fully prior to beginning use of the assay kit.
• Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
• Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
• For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
• Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

• Microplate reader capable of reading absorbance at 450 nm.
• Automated plate washer (optional).
• Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
• Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
• New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
• Absorbent paper or paper toweling.
• Distilled or deionized ultrapure water.

7. Technical Application Tips

• Do not mix or substitute components from other kits.
• To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
• Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
• Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
• Replicate wells are recommended for standards and samples.
• Cover microplate while incubating to prevent evaporation.
• Do not allow the microplate wells dry at any point during the assay procedure.
• Do not reuse tips or tube to prevent cross contamination.
• Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
• Completely remove of all liquids when washing to prevent cross contamination.
• Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
• Equilibrate all materials to ambient room temperature prior to use (standards exception).
• Pipetting less than 1 µL is not recommended for optimal assay accuracy.
• Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
• Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
• Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
• TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Sample Preparation Guidelines

8.1 Sample Preparation and Storage

• Samples must be tested to determine if the kit is valid.

• Store samples to be assayed at 4°C for 24 hours prior being assayed.

• For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1,000 x g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Cell culture supernatants and other biological fluids** – Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

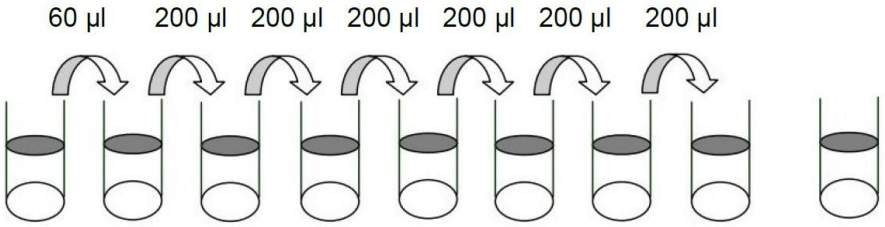
Recombinant Proteins: Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g. antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products

9. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

9.1 Standard

9.1.1 Prepare a fresh standard curve for each assay performed. Reconstituted standards cannot be stored for later use. Briefly spin a vial of standard protein. Add 400 ul **1x Assay Diluent** into standard protein vial to prepare a 500 pg/ml standard stock solution. Dissolve the powder thoroughly by a gentle mix. Add 60 ul of the **D-Dimer standard** from the vial of standard protein, into a tube with 440 ul **1x Assay Diluent** to prepare a 60 pg/ml standard solution. Pipette 400 ul **1x Assay Diluent** into each tube. Use the 60 pg/ml stock solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. **1x Assay Diluent** serves as the zero standard (0 pg/ml)



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	440 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	500 pg/ml	60 pg/ml	20 pg/ml	6.667 pg/ml	2.222 pg/ml	0.741 pg/ml	0.247 pg/ml	0.082 pg/ml		0 pg/ml

9.2 Biotinylated D-Dimer Detector Antibody

9.2.1 Briefly spin the **Biotinylated Detection Antibody** vial before use.

9.2.2 Add 100 µl of **1X Assay Diluent** into the vial to prepare a detection antibody concentrate.

9.2.3 Pipette up and down to mix gently. The detection antibody concentrate should be diluted 80-fold with **1X Assay Diluent**.

9.2.4 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store concentration for future use.

9.3 Streptavidin-HRP Concentrated:

9.3.1 Briefly spin the **Streptavidin-HRP** concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage.

9.3.2 **Streptavidin-HRP** concentrate should be diluted 900-fold with 1X Assay Diluent.

9.4 20X Wash Buffer

9.4.1 If crystals have formed in the **20X Wash Buffer** concentrate, equilibrate to room temperature and mix gently until crystals have completely dissolved.

9.4.2 Dilute 20 ml of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer. Seal and mix gently by inversion. Avoid foaming or bubbles.

9.4.3 Store the **20X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 month. Do not freeze.

9.5 Microplate Preparation

• Micro-plates are provided ready to use and do not require rinsing or blocking.
• Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
• Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9.6 Assay Diluent

- Assay Diluent should be diluted 5-fold with deionized or distilled water before use.
- **1X Assay Diluent** should be used for dilution of serum, plasma, and cell culture supernatant samples.

9.7 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

• Prior to performing the full experiment, test a serially diluted representative sample from your cohort.

- Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

-or-

- Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit

• Mix diluted samples gently and thoroughly.
• Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• Equilibrate all reagents and materials to ambient room temperature prior to use in the procedure.

• Optimal results for intra- and inter-assay reproducibility will be obtained when performing incubation steps at room temperature as indicated below.

10.1 Determine the required number of wells and return any remaining unused wells and desiccant to the pouch.

10.2 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the **D-Dimer Microplate**. At least two replicates of each standard, sample or blank is recommended.

10.3 Cover the plate with the well plate sealer and incubate at room temperature for 2.5 hours with gentle shaking.

10.4 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.5 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.6 Wash 4 times with **1X Wash Buffer**. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or auto washer. 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.

10.7 Add 100 µL of prepared **1X Biotinylated D-Dimer Detector Antibody** to each well.

10.8 Cover with the well-plate sealer and incubate at room temperature for 1 hour with gentle shaking.

10.9 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.10 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.11 Wash plate **4 times** with **1X Wash Buffer** as in Step 10.6.

10.12 Add 100 µL of prepared **Streptavidin HRP Conjugate** into each well, cover with plate sealer and incubate at room temperature for 45 minutes with gentle shaking.

10.13 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle or aspiration.

10.14 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.15 Wash plate **4 times** with **1X Wash Buffer** as in Step 10.6.

10.16 Add 100 µL of **TMB Substrate** to each well, cover with plate sealer and incubate at room temperature in the dark for 30 minutes. Wells should change to gradations of blue. If the color is too deep, reduce the incubation time.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.17 Add 50 µL of **Stop Solution** to each well. Well color should change to yellow immediately. Add the **Stop Solution** in the same well order as done for the **TMB Substrate**.

10.18 Read the O.D. absorbance at 450 nm with a standard microplate reader within 5 minutes of stopping the reaction in step 10.16. If wavelength correction is available, set to 570 nm or 630nm.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

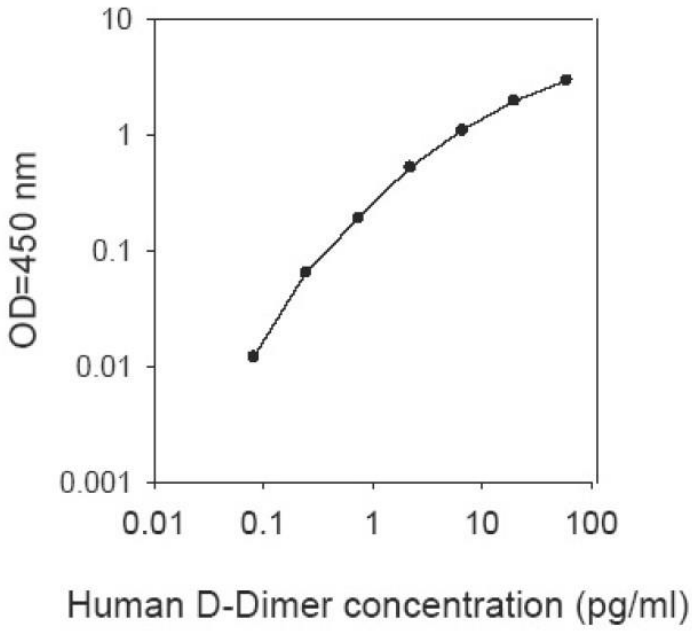
$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

11.1 **Typical Data** – These standard curves are for demonstration only. A standard curve must be run with each assay.



11.2 **Sensitivity** – The minimum detectable dose of Human D-Dimer was determined to be 0.08 pg/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)

11.3 **Spiking and Recovery** – Recovery was determined by spiking various levels of Human D-Dimer into the sample types listed below. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	126.7	108-147
Plasma	124.8	105-141
Cell culture media	106.8	74-124

11.4 Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	106.9	120.3	120.4
	Range (%)	97-118	104-134	112-128
1:4	Average % of Expected	97.93	91.14	122.4
	Range (%)	90-106	83-99	104-141

11.5 Reproducibility – Intra Assay CV%: <10%. Inter-Assay CV%: <12%

11.6 Specificity - This ELISA antibody pair detects human D-Dimer. Other species not determined.

12. Technical Resources

Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

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