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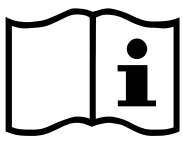
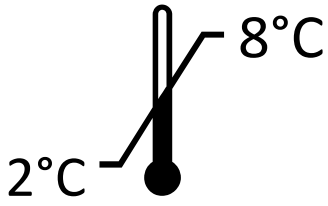
IMUCLONE™ D-Dimer ELISA

REF 602

For Research Use Only





INTENDED USE

The IMUCLONE™ D-Dimer ELISA allows for the quantitative measurement of D-dimer, crosslinked fibrin degradation product (XL-FDP), in human plasma.

For Research Use Only – Not for use in diagnostic procedures.

EXPLANATION OF THE TEST

During early clot formation, thrombin is activated, cleaving fibrinopeptides from the soluble plasma protein, fibrinogen. Molecular polymerization occurs with the formation of soluble fibrin that is then stabilized with covalent cross-linking by FXIIIa activity to produce an insoluble fibrin clot. This stabilized fibrin network is immediately degraded by the fibrinolytic enzyme, plasmin, which process is known as fibrinolysis. Under normal physiological conditions, excess plasmin is rapidly neutralized by alpha-2-antiplasmin within the region of the clot. A variety of XL-FDPs are formed, depending on the extent of fibrinolysis. The smallest fragment is the plasmin resistant species, D-dimer. Detection of D-dimer therefore indicates this sequence of events: thrombin activation, clot formation and subsequent clot lysis.

PRINCIPLE OF THE METHOD

The IMUCLONE D-Dimer ELISA uses a monoclonal antibody against human D-dimer coated to plastic microwells. During an incubation period, D-dimer in the test sample binds to the antibody coated microwells. Following a step during which extraneous plasma proteins are washed away, a horseradish peroxidase (HRP) conjugated monoclonal antibody recognizing the bound D-dimer is added, completing the formation of the antibody sandwich complex.

Following another washing step, TMB (3, 3', 3, 5' – tetramethylbenzidine), a substrate, is added to the microwells and its subsequent reaction with the HRP creates a blue colored solution. The enzyme substrate reaction is stopped by the addition of sulfuric acid, turning the solution color yellow. D-dimer levels are quantified by measuring the solution absorbances at 450 nm and comparing the values with those from a standard curve.

REAGENTS

96-Well Microtest Plate pre-coated with anti-(h) D-Dimer; 12 strips of 8 wells in a frame holder plus storage bag with desiccant
2 vials of Sample Diluent, ready to use (50 mL)
3 vials of D-Dimer Calibrator, see flyer for concentration (lyophilized)
1 vial of D-Dimer Plasma Control I, High (lyophilized)
1 vial of D-Dimer Plasma Control II, Low (lyophilized)
3 vials of Anti-(h) D-Dimer-HRP Immunoconjugate (lyophilized)
1 vial of Conjugate Diluent, ready to use (25 mL)
1 vial Wash Solution, 20 fold concentrate (50 mL)
1 vial of TMB Substrate (Peroxidase Substrate) (25 mL)
1 vial of Stop Solution (0.45 M Sulfuric Acid), ready to use (6 mL)

WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2). As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

For Research Use Only. Not for internal use in humans or animals. Do not use the kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the reagents. Do not smoke, eat or drink in areas in which specimens or kit reagents are handled. Do not pipette reagents by mouth. Wear laboratory coat and disposable gloves throughout the test procedure and wash hands thoroughly afterwards. Avoid splashing or aerosol formation.

Conjugate Diluent	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Sample Diluent	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Wash Solution	Warning 	CONT: Mixture of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H-isothiazol-3-one (3:1) H317, P261, P272, P280, P302+P352, P333+P313, P363, P501
Stop Solution	Danger 	CONT: Sulfuric acid H290, P234, P390

Hazard Statements:	H290	May be corrosive to metals.
	H317	May cause an allergic skin reaction.
Precautionary Statements:	P234	Keep only in original container.
	P261	Avoid breathing dust/fume/gas/mist/vapors/spray.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
	P302 + P352	IF ON SKIN: Wash with plenty of soap water.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
	P363	Wash contaminated clothing before reuse.
	P390	Absorb spillage to prevent material damage.
	P501	Dispose of contents/container in consultation with your regional waste disposer

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date printed on the box when properly stored at 2°-8°C. Allow the reagents to warm to room temperature for 30 minutes before use.

1. **Microtest Strips pre-coated with anti-(h)-D-Dimer**: Once removed from the aluminium pouch, the microwell strips must be used within 30 minutes. Unused strips may be stored at 2°-8°C for 4 weeks when sealed in the original pouch with the desiccant present, protected from any moisture, and stored in the provided storage bag.
2. **Sample Diluent**: Supplied ready to use, once opened, the diluent may be used for up to 4 weeks when stored at 2°-8°C.
3. **D-Dimer Calibrator**: Reconstitute each vial with 2 mL of Sample Diluent to generate a standard of "C" ng/mL. See the enclosed flyer for the exact concentration. The calibrator is stable for at least 8 hours at room temperature.
4. **D-Dimer Plasma Control I and Control II**: Reconstitute each vial with 0.5 mL of distilled water. Vortex for 5 seconds or until completely dissolved. Let stand for 15 minutes at room temperature and homogenize before use**.** The controls are stable for 8 hours at room temperature, 24 hours at 2°-8°C or for 2 months at –20°C or below. Concentrations and ranges are indicated on the flyer provided.
5. **Anti-(h)- D-Dimer-HRP Immunoconjugate**: Reconstitute each vial with 7.5 mL of Conjugate Diluent. Shake the vial gently to homogenize the content. Reconstituted conjugate is stable for at least 8 hours at room temperature or at least 4 weeks at 2°-8°C.
6. **Conjugate Diluent**: Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.

7. **Wash Solution**: If solids are present, incubate the vial for 15-30 minutes in a 37°C water bath. Shake the vial and dilute the amount required 1:20 in distilled water (the entire vial is sufficient to prepare 1 Liter of Wash Solution). The Wash Solution may be used for up to 4 weeks after opening when stored at 2°-8°C in its original vial. Diluted Wash Solution may be used for up to 7 days when stored at 2°-8°C.
8. **TMB Substrate (Peroxidase Substrate)**: Supplied ready to use. Once opened, it may be used for up to 4 weeks when stored at 2°-8°C.
9. **Stop Solution (0.45 M Sulfuric Acid)**: It is ready to use. ***Warning***: Sulfuric acid is caustic. Handle with care. Avoid any skin and eye contact. Wear protective glasses and gloves when handling.

SPECIMEN COLLECTION AND PREPARATION

Either citrate or EDTA collected platelet poor plasma may be used for this assay. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 2,500 x g for 20 minutes.
3. Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at –20°C or below for up to 6 months.
4. Frozen plasma should be thawed rapidly for 15 minutes at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

The D-Dimer Controls and test samples must be tested diluted 1:50 in the Sample Diluent. For expected D-Dimer levels >10 µg/mL (>10,000 ng/mL), samples may be assayed at a higher dilution, e.g. 1:100, 1:200 or higher. If low D-Dimer concentrations are expected, samples may be assayed at lower dilutions, e.g. 1:20, 1:10 or 1:5.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

Distilled water
50-300 µL eight channel multi-pipette
0-200 µL, 200-1000 µL single pipettes
Microwell plate reader for reading absorbance at 450 nm
Microwell plate washer (optional)

Preparation of the Standards

Using D-Dimer Calibrator at "C" ng/mL provided, prepare the following standard solutions in the Sample Diluent:

D-Dimer Standard	C ng/mL	C/2 ng/mL	C/4 ng/mL	C/10 ng/mL	C/20 ng/mL	0 ng/mL
Vol. of D-Dimer Calibrator	1 mL	0.5 mL	0.25 mL	0.1 mL	0.05 mL	0 mL
Vol. of Sample Diluent	0 mL	0.5 mL	0.75 mL	0.9 mL	0.95 mL	1 mL

Mix each standard gently to ensure complete mixing. The standard dilutions are stable for at least 6 hours at room temperature.

Remove the required number of microwell strips from the aluminium pouch, for the series of measures to be performed. Place the strips in the frame provided. Add the reagents to the microwells and perform the various assay steps as indicated on the following table:

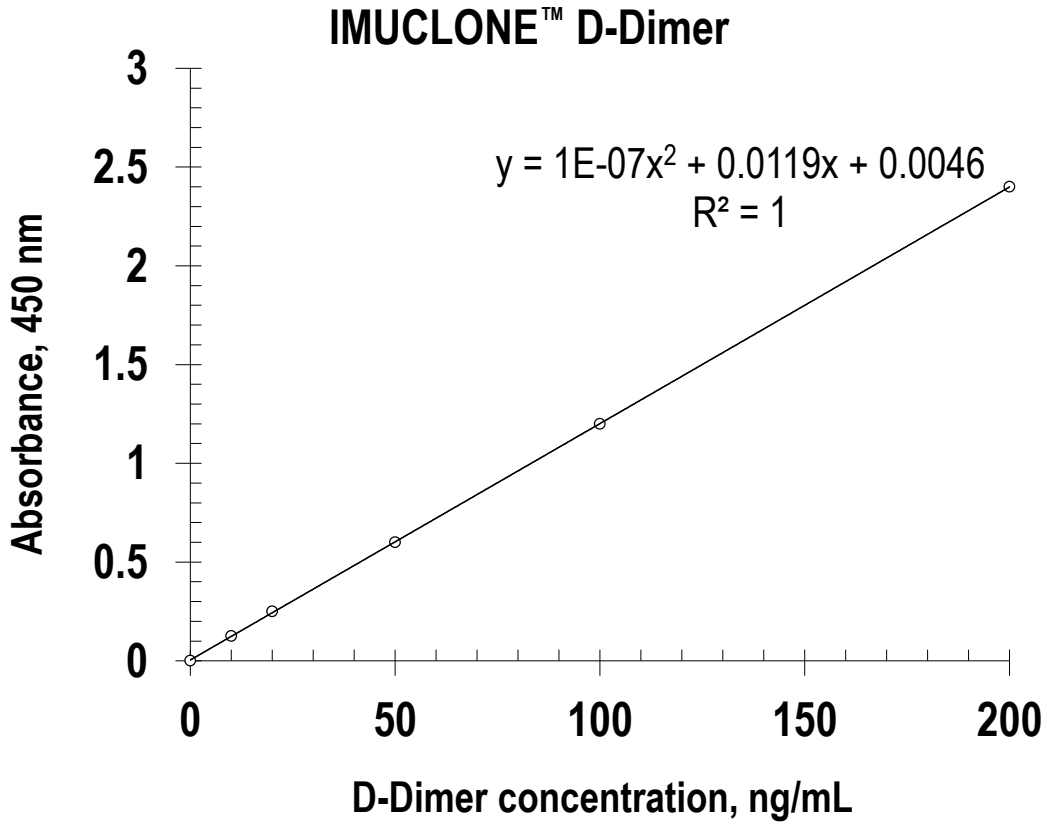
Reagent	Volume	Procedure
D-Dimer standard, diluted test sample or diluted Control	200 µL	Add the standard or diluted test samples to an appropriate microwell.
Incubate for 1 hour at 18°-25°C		
Wash Solution	300 µL	Wash the wells 5 times.
Anti-(h)-D-Dimer HRP Immunoconjugate	200 µL	Add the immunoconjugate to each microwell.
Incubate for 1 hour at 18°-25°C		
Wash Solution	300 µL	Wash the wells 5 times.
TMB Substrate	200 µL	Add the substrate Immediately after washing the wells.
Incubate for exactly 5 minutes at 18°-25°C		
Stop Solution (0.45 M H_2SO_4)	50 µL	Following exactly the same time intervals used for adding the substrate, stop the reaction by adding 0.45 M H_2SO_4.
Wait for 10 minutes to allow the color to stabilize and measure the absorbance at 450 nm.		

Notes:

1. Avoid leaving the plate in the bright sunlight during incubations and particularly during color development.
2. Incubation temperatures of 18°-25°C must be maintained. Temperatures >25°C or <18°C will affect the measured A_{450} to be too high or too low, respectively.
3. Use of a microplate shaker should be limited to 1-2 minutes at the beginning of an incubation step.
4. Do not allow the microwells to dry out between the addition of reagents or following a washing step. Add the next reagent within 3 minutes to prevent the microwells from drying, which could damage the immobilized components. If necessary, fill the microwells with Wash Solution and empty them just before the introduction of the next reagent.
5. When adding the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction.

RESULTS

Construct a standard curve by plotting the mean absorbance value for each D-Dimer standard versus its corresponding concentration in ng/mL. A standard curve should be generated each time the assay is performed. The following standard curve is for demonstration purposes only.



CALCULATIONS

From the standard curve obtained, directly interpolate the D-Dimer concentration in samples tested. The concentration must be multiplied by the actual dilution factor used, i.e. if the sample was diluted by 1:50, multiply the measured concentration by 50. Alternatively, an ELISA software (i.e. Dynex, etc.) can be used for the calculation of concentrations.

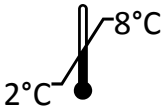
LIMITATIONS OF THE PROCEDURE

Blood activation must be avoided during collection and sample preparation to prevent D-Dimer generation *ex vivo*.

EXPECTED VALUES

The D-Dimer concentration in normal human plasma is usually < 400 ng/mL.

DEFINITION OF SYMBOLS

	Consult instructions for use	LOT	Batch code / Lot number
	Temperature limitation: Store at 2°C to 8°C	REF	Catalog number
	Manufactured by		Use By / Expiration Date
CONT	Contains...		Contains sufficient for <n> tests