

TintElize[®] D-dimer Strip-well format.

Reagents for up to 96 tests.

For Research Use Only

FOR INFORMATION USE ONLY

**Not to be used for performing the assay.
Refer to the insert accompanying kit**

I. INTENDED USE

Biopool TintElize[®] D-dimer is intended for quantitative determination of human D-dimer in plasma by enzyme immunoassay.

II. PRINCIPLE

The Biopool TintElize[®] D-dimer utilises the double antibody principle. Plasma sample or standard containing D-dimer is added to a microtest well, which is coated with a monoclonal antibody, MA-8D3, against D-dimer. After an incubation sufficient to allow >85 % of the D-dimer to bind to the coat antibodies⁷, HRP labelled Fab fragments of anti D-dimer IgG are added. These are allowed to react with the adsorbed D-dimer. The wells are emptied and washed to remove unbound conjugate after which peroxidase substrate (OPD/H₂O₂) is added. The quantity of yellow colour developed is directly proportional to the amount of D-dimer present in the sample.

III. REAGENTS

A. Reagent description

- 1. Microtest strips, 12 pcs:**
Framed 8-well strips, pre-coated, with monoclonal antibody MA-8D3, and prefilled with non-immune mouse IgG and indicator dye.
- 2. Assay Buffer 15X, 2 vials:**
Potassium phosphate, Borate, EDTA, KCl, and Tween 20 buffer solution, pH 7.5 sufficient to make 2 x 0.5 L solution.
- 3. Standard 0 ng/ml, 3 x 0.5 ml:**
Lyophilised, citrated human plasma depleted of D-dimer.
- 4. Standard 1000 ng/ml, 3 x 0.5 ml:**
Lyophilised, citrated human plasma enriched with D-dimer.
- 5. Conjugate, 6 ml:**
HRP labelled Fab fragments of anti D-dimer antibody.
- 6. Substrate, 2 ml:**
Lyophilised *ortho*-phenylenediamine (OPD) with buffer salts.
- 7. Hydrogen Peroxide, 2 ml:**
0.15 % H₂O₂ in water.
- 8. Reagent Reservoirs, 6**
Disposable cardboard trays

B. Reagents preparation.

- 1. Assay Buffer:**
Dilute the contents of 1 vial up to 0.5 L with water. Store diluted assay buffer at 2-8°C for two weeks.
- 2. Standards, 0 and 1000 ng/ml:**
Add 0.5 ml of Assay buffer to each vial, gently agitate for 5 minutes. Mix 200 µl of 0 ng/ml Standard and 200 µl of 1000 ng/ml Standard in a clean vial to obtain a 500 ng/ml Standard. Store tightly capped at 2-8°C for two days.

3. Microtest strips:

After breaking the aluminium foil bag, ensure that the remaining strips are sealed tightly in the bag. Store at 2-8°C for two weeks.

4. Conjugate:

Add 6 ml Assay buffer directly to the conjugate vial and agitate gently for 5 minutes. Store tightly capped at 2-8°C for two weeks.

5. Substrate concentrate:

Add 2 ml of water to the vial and agitate until dissolved (10-15 min.). Store in the dark at -20°C for two weeks.

For one 8-well strip: mix 150 µl of substrate concentrate with 750 µl of water and 150 µl of hydrogen peroxide in a clean container to prepare OPD/ H₂O₂ substrate.

For the complete kit: dilute the 2 ml of concentrate with 10 ml of water, then add all H₂O₂ to the vial. Invert three times to mix.

Important- The OPD/H₂O₂ substrate should be prepared within 30 minutes of use.

- 6. Hydrogen peroxide:** Transfer to a clean capped test tube. Store in the dark at 2-8°C for one month.

IV. STORAGE AND STABILITY

The unopened reagents should be stored at 2-8°C and be used prior to the expiration date. Reconstituted reagents are stable for two weeks if stored as recommended except the standards which are stable for two days if stored as recommended.

V. WARNINGS AND PRECAUTIONS

The Standards are of human origin. Each donor unit of source plasma used in these products has been tested and found negative for Hepatitis B antigens, HIV I and II antibodies, Hepatitis C antibodies, syphilis antibodies and H.T.L.V. I/II antibodies by FDA approved methods. However, because no test method can offer complete assurance that no infectious agents are present, these plasmas should be treated as patient plasma at the Biosafety Level 2, recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/ National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984. All wastes containing biological material should be properly labelled and stored separately from other wastes. Dispose of all waste materials according to prescribed international, national and local regulations.

Potential carcinogen. The substrate (OPD) and Hydrogen Peroxide are harmful and must be handled with care. Avoid ingestion, skin and eye contact. Wear glasses and gloves when handling.

VI. SPECIMEN COLLECTION

Human plasma samples collected with citrate collection tube are recommended. Sample collection procedure: Nine volumes of blood are collected in 1 volume of 0.1 M trisodium citrate, followed by centrifugation at 2500 X g for 15 minutes (NCCLS⁸). The plasmas can be stored for 8 hours at room temperature, 24 hours at 2-8°C or 6 months at -20°C. A single freeze-thaw cycle will not affect assay response. Human plasma collected with EDTA tubes can also be used, please see section E: Limitations of procedure.

VII. PROCEDURE

A. Materials provided

- Microtest strips
- Assay Buffer
- Standard D-dimer 0 ng/ml
- Standard D-dimer 1000 ng/ml
- Conjugate D-dimer
- Substrate (OPD)
- Hydrogen Peroxide
- Reagent Reservoirs.

B. Material required but not provided

- Pipette 8-channel or repeating for 25-100 µl
- Pipettes 1-channel covering 25-1000 µl
- Squeeze bottle
- Paper towels or thin sponge
- Small plastic tubes (2-5 ml)
- Purified water (distilled or deionized and sterile filtered)
- Sulphuric acid H₂SO₄ 1.6 mol/L
- Microtest plate spectrophotometer operable at 492 nm
- Microtest plate shaker with an orbital of 3 mm.

Note Perform all assay steps at ambient (room) temperature, 20-25°C. Temperature equilibrate all reagent solutions.

1. Reconstitution of Microtest wells: Add 50 µl of Assay buffer to each well using a repeating pipette or an 8-channel pipette. Agitate gently for 1 minute.

2. Standard and sample incubation: Add 25 µl of D-dimer standards (0, 500, and 1000 ng/ml) or sample, one addition to each well. The Blue Pre-Fill solution will change colour indicating transfer. Use air displacement pipette, new tip for each transfer. Record the sample positions. Incubate the strip for 30 minutes on a microtest plate shaker at approximately 600 rpm.

3. Conjugate incubation: Add 50 µl of the conjugate to the wells. Use repeating or 8-channel pipette. Incubate the strip for 30 minutes on a micro-test plate shaker at approximately 600 rpm.

4. Wash: Discard the contents and wash the strip four times. Each wash is performed as follows; fill the wells completely with Assay buffer, use a squeeze bottle, empty and "dry" by hitting the strip 4-5 times, face down, against absorbing material (sponge, or paper towels).

5. Substrate incubation: Add 100 µl of OPD/H₂O₂ substrate to each well. Use repeating or 8-channel pipette. The substrate is prepared within 30 minutes before use. Incubate the strip for 15 minutes on a micro-test plate shaker at approximately 600 rpm.

6. Stop: Add 2 ml of concentrated sulphuric acid to 20 ml of water. Store in a glass bottle at room temperature. Stop the enzymatic reaction by adding 100 µl of 1.6 mol/L H₂SO₄. Add in the same order and with the same speed as the substrate was added. Agitate the strip for 5 minutes on a micro-test plate shaker to allow complete mixing and stabilisation of colour. If stored in the dark the coloured product is stable for at least 2 hours.

7. Measurement: Read the absorbance at 492 nm in a microtest plate spectrophotometer. "Blank" the microtest plate reader against air. Calculations: Plot A₄₉₂ against 0, 500, and 1000 ng/ml. Fit a straight line to the points by a minimal least square procedure, e.g. simple curve fit to Delta Graph[®] scatter plot. Use the linear function to calculate the D-dimer values of the samples.

VIII. CALIBRATION

Plot A₄₉₂ against the quantity of D-dimer in the standards. Fit a straight line to the points by a minimum least squares procedure. The D-dimer antigen in the patient's plasma specimen can be determined by interpolation from the standard curve. Note that the slope of the standard curve can show some variation between assays. Users must construct a standard curve each time the assay is performed. See sample standard curve

IX. QUALITY CONTROL

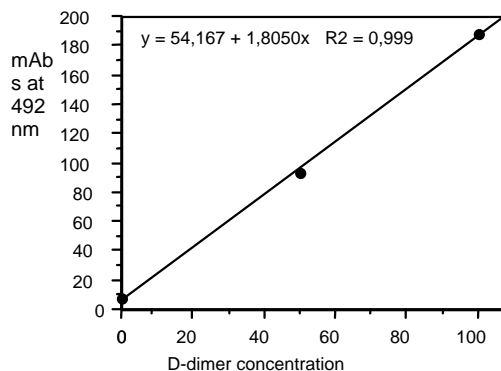
It is recommended to use a plasma sample containing between 200-400 ng/ml D-dimer, stored at -20°C, in small aliquots, as a quality control standard each time the assay is run. Failure to

obtain D-dimer levels within two standard deviations of the mean of the control standard may invalidate the assay.

X. LIMITATIONS OF PROCEDURE

1. Use the purest water available. Bacterial contamination results in peroxidase activity in the water.
2. Use reagents and strips equilibrated to room temperature to minimise "edge effects", which give rise to erroneous absorbance in the peripheral wells.
3. Make certain the plate shaker does not heat the strips. If the top of the shaker feels warm to the hand, cover with a 1 cm thick sheet of insulation (e.g. Styrofoam[®]).
4. It is extremely important to remove unbound conjugate before adding the substrate. Be sure that the wash volume completely fills the wells, and that the wells are completely emptied after each wash. Do not leave the empty wells to dry out, fill directly with the next solution.
5. Samples that contain more than 1000 ng/ml D-dimer should be diluted 1:2 or more with Assay buffer and retested.
6. Some D-dimer generation may occur during storage in EDTA plasmas, these plasmas can be stored no more than 4 hours at room temperature, 8 hours at 2-8°C or 2 months at -20°C.

A. Sample standard curve



XI. EXPECTED VALUES

Healthy adults display a mean plasma D-dimer level of approximately 39 ng/ml⁷. The upper reference limit, 97.5 percentile, is approximately 130 ng/ml⁷. Elevated D-dimer levels are common in pathological conditions such as disseminated intra-vascular coagulation (DIC), pulmonary embolism (PE), deep venous thrombosis (DVT), pre-eclampsia (pre-EC) and sickle cell crisis¹⁻⁵.

XII. PERFORMANCE CHARACTERISTICS

A. Precision

For plasma samples, the intra-assay (within run) and inter-assay (between run) precision is approximately 4% C.V. at 200 ng/ml: at 360 ng/ml both intra- and inter-assay precision is approximately 3% C.V.

B. Accuracy

The accuracy of the TintElize[®] D-dimer kit was shown in a study of 48 patient plasma samples which were also tested with the semi-quantitative Biopool Minutex[®] D-dimer latex. A linear regression coefficient of 0.92 was found.

C. Sensitivity

The test measures D-dimer antigen in the range 0 to 1000 ng/ml. Maximum sensitivity of the assay is 40 ng/ml sample.

D. Specificity

The test is specific for D-dimer by virtue of the screening method used for hybridoma selection³. A hybridoma, secreting antibodies that reacted positively with purified D-dimer but not

with whole fibrinogen or fragment D of fibrinogen, was selected. No cross-reactivity with fibrinogen or des-AA-fibrinogen was observed.

E. Limitations:

The test is unaffected by rheumatoid factor(s) and antibodies against mouse IgG in the sample. This is due to the use of HRP conjugated Fab fragments as conjugate and a large excess of non-immune mouse IgG in each well⁶.

XIII. REFERENCES

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3. Declerck, P.J. *et. al.*: Fibrinolytic response and fibrin fragment D-dimer in patients with deep vein thrombosis. Thromb. Haemostas. 58: 1025-1029, 1987.
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8. National Committee for the National Laboratory (NCCLS) Standards: Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays, Document H21-A2, Vol. 11, No. 23, 1991

XIV. RISK AND SAFETY



Xn

Harmful – OPD

R20/21/22 Harmful by inhalation, in contact with skin and if swallowed.

R40 Limited evidence of carcinogenic effect.

R52/53 Harmful to aquatic organisms. May cause long-term adverse effects in the aquatic environment.

S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S61 Avoid release to the environment. Refer to special instructions/Safety data sheets.

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