TECHNOZYM® D-Dimer ELISA

For research use only

GB

REF 2599006 TECHNOZYM® D-Dimer ELISA

Symbols key			
	Manufacturer		Expiry date
	Storage temperature		Consult instructions for use
AQUA	Distilled water		Determinations
BUF	Reaction buffer	LOT	Lot
CAL	Calibrator	MTP	Microtiter plate
CONJ	Conjugate	REF	Catalogue number
CONT	Control	RTU	Ready to use
DIL	Dilute or dissolve in	STOP	Stop solution
INC	Incubation buffer	SUB	Substrate
RUO	For research use only	WASH	Washing solution concentrate

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TECHNOZYM® D-Dimer ELISA

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PRODUCT DESCRIPTION

INTENDED USE

TECHNOZYM® D-Dimer ELISA can be used to determine D-Dimer concentrations in plasma. D-Dimer is generated as a specific soluble degradation product during fibrinolysis. Elevated D-Dimer levels are found in cases of disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT) and pulmonary embolism (PE) but other circumstances may also lead to high D-Dimer levels such as old age, pregnancy, cancer, liver disease and infection.
The ELISA system has a higher sensitivity when compared with latex agglutination tests.

COMPOSITION

1. ELISA test strips (12), with 8 wells each, coated with anti D-Dimer monoclonal antibody; the drying agent is supplied in an aluminium bag.
2. Washing buffer concentrate (PBS; pH 7.3); containing detergent; 0.01 merthiolate; 1 bottle, 80 mL.
3. Incubation buffer (PBS; pH 7.3); contains stabiliser protein; 0.05% proclin; and blue dye, 1 bottle, 90 mL, ready for use.
4. Calibrators (Standards) numbered 1-5; lyophilised, 1 vial each. **Concentrations are lot-dependent; consult label on the vial.**
5. Control plasmas "low level" and "high level", lyophilised; 1 vial each. **Concentrations are lot-dependent; consult the label on the vial.**
6. Conjugate monoclonal Anti-D-Dimer-POX; dyed blue; 1 vial, 0.3 mL.
7. Chromogen TMB (tetramethylbenzidine); 1 bottle, 12 mL; ready for use.
8. Stopping solution: sulphuric acid 0.45 mol/L; 1 bottle 12 mL; ready for use.
9. Adhesive film for ELISA test strips (2).

MATERIAL REQUIRED (but not supplied with the kit)

1. Distilled water
2. Test tubes for diluting standard and samples
3. Measuring cylinder (1000 mL)
4. Precision pipettes (10, 100 and 1000 µL)
5. Variable pipette (1000 µL)
6. Multichannel and/or dispensing pipettes (100 and 200 µL)
7. ELISA washer or multichannel pipette
8. ELISA reader with 450 nm filter, with a 620 nm reference filter if available.

WARNING AND PRECAUTIONS

- For research use only
- All human blood or plasma products as well as samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control plasmas made from human blood and any individual plasma involved in the procedure is HB_sAg, HIV 1/2 Ab and HCV-Ab-negative (see labels on kit and/or bottles).
- Stopping solution (sulphuric acid) may irritate the skin. Should acid get into your eyes, wash out immediately with water and consult a doctor.
- The reagents sometimes contain preserving agents (merthiolate). Beware of swallowing! Avoid contact with skin or mucous membranes

STABILITY AND STORAGE

The expiry date printed on the labels applies to storage of the unopened bottles at + 2…8 °C.
Stability after reconstitution/opening:

Material/Reagent	State	Storage	Stability
Calibrators, control plasmas	after reconstitution	-20 °C	6 months
ELISA test strip	after opening	2 ... 8 °C with adhesive film in plastic bag with drying agent	expiry date
Washing buffer conc.	after opening	2 ... 8°C	6 months
Washing buffer	1+11.5 dilution of concentrate	2 ... 8 °C	3 weeks
Incubation buffer (= sample dilution buffer)	after opening	2 ... 8 °C	2 months
Conjugate	after opening	2 ... 8 °C	6 months
	working solution	room temperature	60 minutes
Chromogen TMB	after opening	2 ... 8 °C	expiry date

TEST PROCEDURE

PREPARATION OF SAMPLES

Sample material: Plasma.
Citrated or EDTA plasma should be used. Centrifuge for 15 minutes at a minimum of 2500 g (DIN 58905). The plasma sample may be stored for 3 hours at room temperature; otherwise the sample ought to be frozen immediately after centrifugation. Stable at -20°C for 6 months. Avoid repeated freeze-thaw cycles. Use of serum samples is not recommended.

PREPARATION OF REAGENT

1. Before starting the test, all the required components are to be brought to room temperature.
2. Preparing the washing buffer: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (Diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37°C within 10 minutes.
3. Reconstituting calibrators and control plasmas: Calibrators and control plasmas are reconstituted with 0.5 mL distilled water and mixed for 10 seconds after a reconstitution time of 15 minutes (vortex mixer). Reconstituted components are clear to slightly turbid. Control plasmas, normal and low level samples are used undiluted. For high level samples (up to 15-20 µg/mL) a dilution of 1:20 (20 µL plasma + 380 µL incubation buffer) is recommended, for abnormally high samples dilute 1:200 (40 µL plasma +360 µL incubation buffer).
4. Preparing the conjugate working solution (1+50): Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer

For 8 test wells: Mix 20 µL conjugate with 1000 µL incubation buffer

PERFORMANCE OF THE TEST

SAMPLE INCUBATION (reference 1,2)	Pipette **calibrators, control plasmas**, **diluted samples** into test wells; cover test strips with film	**100 µL**
	Incubate at **37°C**	**60 minutes**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
CONJUGATE REACTION (reference 1,2)	Pipette **conjugate working solution** into wells, cover test strip with film	**100 µL**
	Incubate at **37°C**	**60 minutes**
WASHING (reference 1,3,4)	**Washing buffer**	**3 x 200 µL**
SUBSTRATE REACTION (reference 1,2)	Pipette **Substrate solution** into test wells, cover test strip with film	**100 µL**
	Incubate at **room temperature**	**10 minutes**
STOPPING (reference 1,2)	Pipette **stopping solution** into wells	**100 µL**
MEASURING (reference 5)	ELISA-Reader, **450 nm**	shake 10 sec., measure within 10 min.

References

1. Reagents of different lots must not be combined
2. Precision and performance, among others, essentially depend on the following factors:
   - Thorough mixing of all substances used for dilution, 10 sec. with Vortex Mixer
   - Test calibrators, controls and samples in duplicates
   - Incubate at indicated temperature (RT: room temperature, 20...25°C)
   - Strict observance of the order of pipetting and of the time element as indicated
   - The time for sample incubation, conjugate and substrate reaction as indicated starts after pipetting the last sample. Incubation times should not vary by more than □ 5%.
   - During sample incubation and conjugate reaction, the time for pipetting calibrators/control plasmas/samples and/or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).
   - During substrate reaction and at stopping, the time needed for pipetting the substrate and/or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel- and dispensing pipettes.
3. Label/number strips with a water resistant pen in case the strips accidentally fall out of the frame during testing.
4. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper; by gentle tapping, the last remnants must be removed.
5. By measuring the difference in wave lengths at 450 and 620 nm the precision of the test is increased.

LIMITATION OF THE TEST

The test cannot be used with serum samples. Interference with other Fibrin degradation products (FDP) can be mostly excluded as the antibody used is specific for a neo-antigen on the D-Dimer structure.

ANALYSIS RESULTS

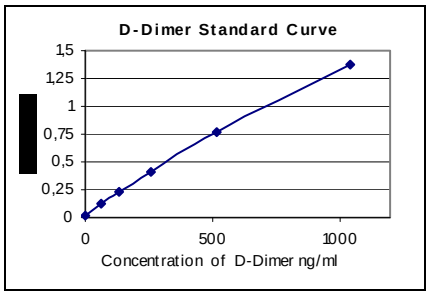
CALCULATION OF THE RESULTS

Setting up a reference curve: X axis: Concentration D-Dimer ng/mL
Y axis: Extinction
Graph plot is linear-linear with a linear, point to point fit or cubic spline.

Assessment of reference curve

- The extinction coefficient of the highest calibrator should be between 1.0 and 2.5.
- The validity of the test may be checked on the basis of the calculated control values.

Example of standard curve



Measuring concentration of samples

- Read off the concentration from the reference curve.
- If there are samples with extinction coefficients higher than that of the highest point on the curve, they have to be prediluted with incubation buffer. The measured concentration then has to be multiplied with the dilution factor.

REFERENCE RANGE

Normal range for D-Dimer was determined between 0 – 250 ng/mL (n = 148).
It is recommended that individual laboratories establish their own normal range.

STANDARDISATION

There is no international standard for D-Dimer. Technoclone's standard was calibrated against the standard of a commercial D-Dimer Latex assay.

LITERATURE

1. Nieuwenhuizen W. A reference material for harmonization of D-Dimer assays; SSC Communication. Thromb Haemostas 1997; 77: 1031-3