Diazyme Laboratories
12889 Gregg Court
Poway, CA 92064, USA
Tel: 858-455-4768 / Fax: 858-455-3701
Email: **support@diazyme.com**
Website: www.diazyme.com

Diazyme D-Dimer Assay

Configuration
The Diazyme D-Dimer Assay reagent is provided in packaging configuration:

Catalog No.	Kit size
DZ179A-K	R1: 1 x 20 mL R2: 1 x 8 mL

* Calibrators and controls sold separately

Intended Use
The D-Dimer Assay is for the quantitative determination of fibrinogen/fibrin degradation products (D-Dimer) in human plasma. Measurement of D-Dimer is used as an aid in detecting the presence of intravascular coagulation and fibrinolysis. For *in vitro* diagnostic use only.

Background
Thrombus formation is normally followed by an immediate fibrinolytic response. The resultant generation of plasmin causes the release of fibrin degradation products (predominantly containing D-Dimer) into the circulation[1].

Test Principle
Diazyme's D-Dimer Assay is based on a latex enhanced immunoturbidimetric assay. D-Dimer proteins in the sample bind to the specific anti-D-Dimer antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of D-Dimer in the sample. The Roche Modular P calculates the D-Dimer concentration of a patient specimen by interpolation of the obtained signal of a 6-point calibration curve.

Reagent – Working Solutions
Reagent 1
100 mM Tris-buffer pH 8.2 solution

Reagent 2
Suspension of anti-human D-Dimer mouse monoclonal antibody coated latex particles (0.2%)

Precautions
1. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
2. Do not use the reagents, calibrator, and controls after the expiration date labeled on the outer box. The assay should be recalibrated and controls run with each new lot of reagents.
3. Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories (HHS Publication Number [CDC] 93-8395). Avoid ingestion and contact with skin and eyes.
4. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768.
5. Do not mix reagents of different lots.

Warnings
The reagent contains <0.1% sodium azide, NaN_3, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent azide buildup.

Reagent Handling
1. R1 ready for use
2. R2 mix reagent gently before use and once weekly thereafter.

Reagent Stability and Storage
D-Dimer assay reagents, calibrators, and controls should be stored at 2-8°C. **DO NOT FREEZE**. The reagents, calibrators, and controls are stable when stored as instructed until the expiration date on the label. Open reagent bottles are stable for 4 weeks if stored on board the refrigerated compartment of the Roche Modular P analyzer.

Specimen Collection and preparation
Plasma samples with 0.109 Molar (~3.2%) Na Citrate concentration can be used for the D-Dimer assay. Mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate plasma as soon as possible after collection. Samples can be stored at 2-8°C, if analyzed within 4 days, and 3 months when stored at -20°C. When thawing frozen samples, thaw at room temperature and mix thoroughly before use. Once thawed, a sample may not be refrozen for analysis.

Materials Provided
See "Reagent – Working Solutions" section for reagents.

Materials Required (but not provided)
- Controls for validating the performance of the Diazyme D-Dimer Assay are provided separately (Cat. No. DZ179A-CON)
- Calibrators for the Diazyme D-Dimer assay are provided separately (Cat. No. DZ179A-CAL)
- 0.9% Saline is needed as Calibrator 0
- General laboratory equipment
- Roche Modular P instrument

Assay Procedure
Refer to Roche Modular P application sheet for step by step instructions.

Calibration
0.9% saline and five levels of D-Dimer calibrator (Cat. No. DZ179A-CAL) are needed for calibration. The lot specific calibrator values are stated in the Certificate of Analysis. Refer to Roche Modular P application sheet for step by step instructions.

Calibration frequency
The Diazyme D-Dimer assay calibration stability is 4 weeks on Modular P analyzers. Additionally, the assay should be recalibrated and controls run with each new lot of reagents. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Quality Control
We recommend that each laboratory use the Diazyme D-Dimer Control Set, listed under Materials Required section, to validate the performance of D-Dimer reagents. The D-Dimer Control Set is available from Diazyme Laboratories (Cat. No. DZ179A-CON). The control interval and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Each laboratory should follow federal, state, and local guidelines for testing QC material.

Results
Results are printed out in µg/mL. The Diazyme D-Dimer Assay result unit is µg/mL FEU (Fibrinogen Equivalent Units).

Reference Range[2]
The reference interval was established to be < 0.50 µg/mL FEU. However, each laboratory is recommended to establish a range of normal values for the population in their region.

Limitations

1. Diazyme D-Dimer Assay is not intended to be used for exclusion of VTE.
2. Diazyme D-Dimer Assay has not been established in pediatric subjects.
3. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Diazyme D-dimer Assay has been formulated to minimize this interference; however, specimens from patients who have been routinely exposed to animals or to animal serum products may contain heterophilic antibodies which may cause erroneous results.
4. As with any latex turbidimetric immunoassays, Diazyme D-Dimer Assay runs should be followed with appropriate and thorough wash steps. Please consult instrument manuals for further information.
5. Diazyme D-Dimer Assay reagents, calibrators, and controls should be stored at 2-8°C. **DO NOT FREEZE**.

Performance Characteristics

The data (precision, method comparison, linearity, LOB/LOD/LOQ and interference) determined using Roche Modular P is given below.

Precision

Internal Study

The precision of the Diazyme D-Dimer Assay was evaluated according to Clinical Laboratory Standards Institute EP5-A guideline.

In the study, three levels of pooled citrated plasma specimens containing 0.60 µg/mL, 2.41 µg/mL and 5.88 µg/mL FEU, respectively. The low plasma sample was unaltered. The other two plasma samples were spiked with D-Dimer stock solution to targeted concentrations and assayed. Three levels of D-Dimer controls containing 0.97, 2.99 and 7.47 µg/mL FEU, respectively were also tested with 2 runs per day with duplicates over 20 working days with three lots of reagent and three lots of calibrators. The combined results are shown below:

Plasma Samples Within Run Precision

	Level 1	Level 2	Level 3
N	240	240	240
Mean (µg/mL FEU)	0.60	2.41	5.88
SD	0.03	0.05	0.08
CV%	5.0%	2.0%	1.4%

Plasma Samples Total Precision

	Level 1	Level 2	Level 3
N	240	240	80
Mean (µg/mL FEU)	0.60	2.41	5.88
SD	0.04	0.07	0.19
CV%	6.2%	2.7%	3.2%

Control Samples Within Run Precision

	Level 1	Level 2	Level 3
N	240	240	240
Mean (µg/mL FEU)	0.97	2.99	7.47
SD	0.03	0.05	0.11
CV%	2.9%	1.6%	1.4%

Control Samples Total Precision

	Level 1	Level 2	Level 3
N	240	240	240
Mean (µg/mL FEU)	0.97	2.99	7.47
SD	0.04	0.08	0.27
CV%	4.4%	2.8%	3.6%

External Study

The precision of the Diazyme D-Dimer Assay was also evaluated at three external sites by intended users. In the study, four different patient samples of citrated plasma containing 0.36 µg/mL, 1.06 µg/mL, 3.53 µg/mL and 7.20 µg/mL FEU respectively, were tested in duplicates with 2 runs per day over 5 nonconsecutive working days using three lots of reagent, three lots of calibrators, three different clinical testing sites, three different operators and three different instruments. The results are shown below:

Sample	within run	between run	between day	between lot	total	Mean µg/mL FEU
	CV%	CV%	CV%	CV%	CV%	
1	8.9%	8.3%	7.6%	7.1%	11.5%	0.36
2	3.8%	3.6%	7.4%	8.3%	8.3%	1.06
3	2.9%	3.9%	3.1%	0.7%	4.7%	3.53
4	1.6%	3.1%	2.4%	1.5%	3.5%	7.20

LOB, LOD, and LOQ

The LOB, LOD, LOQ of the Diazyme D-Dimer Assay was determined according to CLSI EP17-A. LOB was determined to be 0.06 µg/mL FEU; LOD was determined to be 0.09 µg/mL FEU. To determine LOQ, specimens with mean measured concentrations ranging from 0.02 to 0.93 were assayed. Based on the EP evaluator-8 fitted model, the LOQ (lowest concentration for which CV is less than a target of 20%) is 0.15 µg/mL FEU.

Linearity

Eleven levels of the D-Dimer linearity set were prepared by diluting a specimen containing 8.0 µg/mL FEU with saline according to Clinical and Laboratory Standards Institute EP6-A and tested on Modular P. Diazyme D-Dimer assay is linear from 0.15 to 8.0 µg/mL FEU.

Method Comparison

To demonstrate accuracy, the Diazyme D-Dimer Assay was evaluated by testing individual citrated plasma from the intended target population (Intensive Care Unit, Obstetrics, Trauma, Post-Operative and Operating Room) with comparison to a legally marketed D-Dimer device at the manufacturer site and two external clinical laboratories. A total of 128 citrated plasma samples (88 unique samples) with D-Dimer ranging from 0.17 to 7.95 µg/mL FEU were compared. Repeat testing was addressed by bootstrapped regression analysis with stratification is shown below:

Parameter	**Total of 3 sites**
Slope	0.979
95% CI	0.909 to 1.060
Intercept	-0.106
95% CI	-0.260 to 0.026
R^2	0.939

The bias around the medical decision point is -0.12 µg/ml FEU

Interference

The following substances do not interfere with this assay at the levels tested (less than 10% bias):

Hemoglobin:	up to 500 mg/dL
Bilirubin:	up to 40 mg/dL
Bilirubin Conjugated:	up to 40 mg/dL
Triglycerides:	up to 1000 mg/dL
Ascorbic acid:	up to 176 mg/dL
Rheumatoid Factor	up to 100 IU/mL
Heparin	up to 1.5 IU/mL
HAMA	up to 490 ng/mL

References

1. BJH Guideline. British Journal of Haematology. 124, 15-25.
2. Alan H.B. Wu. Tietz Clinical Guide to Laboratory Tests. Fourth Ed. Saunders Elsevier, 11830 Westline Industrial Drive, St. Louis, Missouri 63146. 2006; 328-329

EC REP
MDSS
Schiffgraben 41
30175 Hannover, Germany

12889 Gregg Court
Poway, CA 92064, USA
Tel: (858) 455-4754
Fax: (858) 455-4750