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REAGENT 5 x 1 mL

IVD CE

DIAGON LTD.
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HUNGARY

Dia-DEF IX

- **Yumizen G405**
- **Yumizen G800/G800h/G850h**
- **Yumizen G1500/G1550/G1500h/G1550h**

PTT based Factor Deficient Plasma for *in vitro* diagnostic use.

Intended Use

The **Dia-DEF IX** factor deficient plasma is intended for the quantitative determination of Factor IX with activated partial thromboplastin time (PTT) reagent and one-stage coagulation test in decalcified plasma on automated, semi-automated and/or manual coagulometric methods, for *in vitro* diagnostic use for all human populations.

Clinical Interest (1, 2, 3, 4, 5, 6)

The decreased level of factors in common and extrinsic pathways (Factor IX) could be observed in the following cases:

- Congenital or acquired factor deficiency states
- Liver disease
- Autoimmune haemohilia states

Method

The measurement is based on the fact that normal plasma can correct the prolongation of the activated partial thromboplastin time (PTT) of Factor IX deficient plasma.
Consequently, the PTT of the mixture of deficient plasma and normal plasma is shorter than the PTT of deficient plasma.
The higher the Factor IX level of the plasma used for mixing, the greater the correction is, so the measured PTT is shorter.
In this way, from the mixture of deficient plasma and different dilutions of calibrator, a Second (PTT) - Percentage (Factor IX) calibration curve can be recorded, so then Factor IX percentage (%) level of tested sample can be determined from the clotting time of the mixture of deficient plasma and tested plasma.

Handling

1. Allow the vial to stand for at least 5 min (20 - 25°C) before reconstitution.
2. Reconstitute the content of one vial with 1 mL of deionized or purified water.
   Be careful when opening the rubber cap as some lyophilized material may be lost.
3. Replace the cap and gently invert the bottle (5 - 10 times) to disperse the contents (avoid foaming).
4. Allow the vial to stand for at least 30 min (20 - 25°C).
5. Gentle horizontal mixing is recommended during reconstitution.
6. Swirl the vial horizontally gently several times (5 - 10 times) before using it, but do not shake.

For optimal performance remove the reagent from the instrument after use, close the vial and store at 2 - 8°C.
Each laboratory must establish the quality assurance procedures to be followed. These must conform to the current accreditation requirements and pertinent regulations.

Care should be taken not to interchange the caps with others products.

Calibrator

The factor assays reported dimension with **Dia-DEF IX** factor deficient plasmas is calculated from a log-log point to point calibration curve.

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Each laboratory should prepare a lot specific calibration curve, use:

- **Dia-CAL** (95012 / 1300130703) (not included)
  12 x 1 mL (lyophilisate)
- Buffer solution: **Yumizen G IMIDAZOL** (1300036385) (not included)
  12 x 15 mL
- **Yumizen G CaCl2 4** (1300036386) (not included)
  12 x 4 mL
- **Yumizen G APTT 4** (1300036377) (not included)
  2 x 4 mL

Calibration Procedure for Semi-Automated Analyzers

- Yumizen G405

The calibration is a calibrator dilution based process that can be used with HORIBA analyzers (Yumizen G Line). Duplicated measurement is recommended.

1	Prepare different calibrator dilutions with Yumizen G IMIDAZOL: 1:3, 1:5, 1:10, 1:20, 1:40, 1:80.	
2	Add the diluted calibrator into the cuvette.	25 µL
3	Add factor deficient plasma into the cuvette.	25 µL
4	Incubate at 37°C.	120 s
5	Add **Yumizen G APTT 4** reagent into the cuvette.	50 µL
6	Incubate the plasma with **Yumizen G APTT 4** reagent.	300 s
7	Add **Yumizen G CaCl2 4** start reagent into the cuvette.	50 µL
8	Simultaneously start the timer.	~ 3 min
9	Prepare a calibration curve from the results (second and relevant % derived from calibrator value according to dilution).	

Calibration Procedure for Fully Automated Analyzers

- Yumizen G800/G800h/G850h
- Yumizen G1500/G1550/G1500h/G1550h

The factor assays calibration is automatically prepared when using fully automated coagulation analyzer according to the test setup of the instrument for the assay.

Precautions of calibration

In case of determination by any other hemostasis analyzers, please follow the instructions of the manual.
Every factor determination requires local calibration with the given lot of reagent on the given instrument.

Control

For internal quality control, use:

- **Dia-CONT I-II** (91020 / 1300130704) (not included)
  2 x 10 x 1 mL (lyophilisate)

The frequency of controls and the confidence intervals should correspond to laboratory guidelines and country-specific directives. You should follow federal, state and local guidelines for testing quality control materials. The results must be within the range of the defined confidence limits. Each laboratory should establish a procedure to follow if the results exceed these confidence limits.
Each control should be assayed daily and/or after a calibration.

Materials Required but not Provided

- HORIBA analyzers (Yumizen G Line) are recommended.
- Third-party analyzers can also be used, where the clotting based method and free test setup access are allowed.
- Calibrator: **Dia-CAL** (95012 / 1300130703)
- Control: **Dia-CONT I-II** (91020 / 1300130704)
- **Dia-DEF IX** is recommended with: **Yumizen G APTT 4** (1300036377).
- Buffer solution: **Yumizen G IMIDAZOL** (1300036385)
- **Yumizen G CaCl2 4** (1300036386)
- Distilled water
- Standard laboratory equipment

Specimen (7)

The factor assays with **Dia-DEF IX** factor deficient plasmas requires freshly decalcified plasma.
Carefully mix 1 part anticoagulant solution with 9 parts venous blood in primary tube, avoiding the formation of foam.
Recommended anticoagulant:

- 3.2% (109 nmol/L) dihydrate form of trisodium citrate.
- 3.2% (109 nmol/L) dihydrate form of trisodium citrate buffered with theophylline, adenosine and dipyridamole-CTAD.

Centrifuge the blood specimen at 1500 g for no less than 15 min at room temperature.
Store it in an unopened tube at room temperature.
Do not store on ice or at +2 to +8°C as cold activation of FVII, or a gradual loss of FVIII may alter results .
Plasma should be tested within 4 h of blood collection.

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Frozen samples for thawing should not stand at 37°C for more than 5 min.
For additional information, please refer to CLSI document H21-A5.

Specimen Stability		
T (°C)	20 - 25	-22 to -26
Factor IX	4 h	6 months

Semi-Automated Analyzers Procedure

The factor assays with **Dia-DEF IX** factor deficient plasma can be used with semi-automated coagulation analyzers based on the method detailed below.
Duplicated measurement is recommended.

1	Dilute the sample with Yumizen G IMIDAZOL.	1:5
2	Add the diluted sample into the cuvette.	25 µL
3	Add factor deficient plasma into the cuvette.	25 µL
4	Incubate at 37°C.	120 s
5	Add **Yumizen G APTT 4** reagent into the cuvette.	50 µL
6	Incubate the plasma with **Yumizen G APTT 4** reagent.	300 s
7	Add **Yumizen G CaCl2 4** start reagent into the cuvette.	50 µL
8	Simultaneously start the timer.	~ 3 min

Precautions of test procedure

In case of determination by any other hemostasis analyzers, please follow the instructions of the manual.

Reference Range (8)

Each laboratory should establish its own reference ranges.
The values given here are used as guidelines only.
Normal adult range: 60% to 150%.

Storage and Stability

Stability before opening

Stable up to the expiry date on the label if stored at 2 - 8°C.

Stability after reconstitution

	20 - 25°C	15 - 19°C (on board)	2 - 8°C
Dia-DEF IX	8 h	8 h	8 h

Do not freeze.

Waste Management

- Please refer to local legal requirements.
- This product contains less than 1.0 g/L of sodium azide as a preservative. Sodium azide may react with lead and copper to form explosive metal azides.

Expected Results

The factor assay results can be reported in the following units:

- **Second:** observed clotting time of the sample.
  This value is not informative itself, as a final result.
- **Percentage:** proportional part of the normal factor activity, which is calculable from the calibration curve.

Precautions of Calculation

- By calculating with inappropriate data or using the supplied data improperly, erroneous results can be obtained.

General Precautions

- This product is for professional *in vitro* diagnostic use only.
  For laboratory use.
- For prescription use only.
- This reagent is classified as non-hazardous in compliance with regulation (EC) N°.1272/2008.
- **Warning:** Human source material. Each donor unit used in the preparation of this product tested with HBsAg, anti-HIV 1-2, anti-HCV, anti-TP screening tests and found to be non-reactive. Consequently, it should be treated as potentially infectious and handled with the appropriate precautions.
- Do not pipette by mouth.
- Do not replenish the products.
- Do not swallow. Avoid contact with skin and mucous membranes.
- Observe the standard laboratory precautions for use.

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- The product vials should be discarded after use. Disposal of all waste material should be in accordance with local guidelines.
- Please refer to the SDS associated with the product.
- Do not use the product if there is visible evidence of biological, chemical or physical deterioration.
- Do not use the product if the recommended storage conditions, including temperature, are not followed.
- User must be trained by a HORIBA representative before attempting to operate the device.
- It is the user's responsibility to verify that this document is applicable to the product used.
- For technical assistance, you can call +33 (0)4 67 14 15 16.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the country in which the user and/or the patient is established.
- Using of third-party hemostasis analyzers may cause a risk of system un-harmonization.
- It is the user's responsibility to evaluate the risk of using a third-party hemostasis analyzers.

Performance (9, 10, 11)

The performance data listed below are representative of performance on HORIBA systems.

Precision

Repeatability (on automated analyzers) [a]

The repeatability tests with Yumizen G APTT 4 on Yumizen G Line instruments give the following results (data obtained on internal study).

- 2 controls (10 runs)

	Mean value %	CV %
Control specimen 1	76.4	3.895
Control specimen 2	45.5	6.208

Maximum acceptance criteria (CV %): < 8%.

Reproducibility (on automated analyzers) [b]

The reproducibility tests with Yumizen G APTT 4 on Yumizen G Line instruments give the following results (data obtained on internal study).

- 2 controls (10 runs)

	Mean value %	CV %
Control specimen 1	74.0	5.555
Control specimen 2	37.0	7.184

Maximum acceptance criteria (CV %): < 15%.

Measuring Range

The measuring range is 1 - 150% on the Yumizen G Line instruments.

Linearity

The linearity range without extra dilution on HORIBA analyzers (Yumizen G Line) is 1 - 150%.

Correlation

The diagnostic utility of **Dia-DEF IX** is validated, using Passing-Bablok regression and Bland et Altman plot procedure on Yumizen G Line analyzers, based on comparison with another device from the market:
Number of samples: 50

- Passing-Bablok regression: 0.930 (slope)
- Bland et Altman plot procedure: -0.484 (difference)

Precautions of Characteristics

The measurement data was generated during a performance evaluation and is not recommended as an acceptance criterion.

Reference

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3. Lei B, Liang C, Feng H. Congenital hemophilia A with low activity of factor XII: a case report and literature review. Ital J Pediatr (2021) **47** 204.
4. Pinchover LB, Alsharif R, Bernal T. Acquired haemophilia a secondary to multiple myeloma: management of a patient with a mechanical mitral valve. BMJ Case Rep (2020) **19** (9): e230798.

[a]Modification: modification of repeatability.
[b]Modification: modification of reproducibility.

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7. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays. Approved Guideline, 5th ed., CLSI (NCCLS) document H21-A5 (2008).
8. Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood (1992) **80** (8): 1998-2005.
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11. Triplett DA, Harms CS. Procedures for the Coagulation Laboratory. Am. Society for Clin. Path, Chicago (1981), **36**.