DIAGON
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INSTRUCTION FOR USE

Dia-F Deficient Plasmas

Factor Deficient Plasmas
(APTT Based - Factors VIII, IX, XI, XII)

CE

Cat.No.: 08305 Dia-FVIII Deficient plasma
Cat.No.: 08405 Dia-FIX Deficient plasma
Cat.No.: 08605 Dia-FXI Deficient plasma
Cat.No.: 08705 Dia-FXII Deficient plasma

Intended use

The Factor Deficient Plasmas are intended for the quantitative determination of the respective factor in patients suspected of having a congenital or acquired deficiency of this coagulation protein. Quantitative measurement of individual coagulation factors by the one-stage method requires substrate plasma lacking the factor to be measured. A dilution of the test plasma is mixed with the factor deficient plasma and the clot time of the mixture determined. The degree of clot time correction with the patient plasma is compared to the correction with a reference material, allowing the % activity of the patient plasma to be determined[1]. The Diagon Factor Deficient Plasmas can be used on any instrument capable of performing APTT-based factor assay testing. Refer to the instrument Operators' Manual for appropriate instructions.

Warnings

The reagents contained in this kit are for *in vitro* diagnostic use only.

Precautions

Potentially dangerous material – **DO NOT INGEST!** Wear gloves when handling all kit components.

Plasma products have been screened and found negative for the presence of Hepatitis B Antigen (HbsAg) HIV 1 and 2 antibody and HCV antibody; however they should be handled with the same precautions as a human plasma sample. All immunodepleted plasmas are HCV negative

Waste Material Treatment

Refer to the product safety data sheets for risk and safety phrases and disposal.

Materials provided

Component	Content	Description	Preparation
Factor Deficient Plasmas	5 x 1 mL	All the Factor Deficient Plasmas listed above are derived from human plasma and contain less than 1% residual factor activity.	Reconstitute each vial with 1 mL of purified water. Swirl gently and allow to stand for 15 minutes. Mix well before use (do not shake).
Each kit contains Instructions For Use.			

Materials required but not provided

Cat.No.: 09605 Dia-CAL Spec
Cat.No.: 41192 Dia-CaCl2
Cat.No.: 21180 Dia-IMIDAZOL
Cat.No.: 71048 Dia-PTT
Cat.No.: 09305 Dia-CONT Spec N
Cat.No.: 09405 Dia-CONT Spec P

Storage and stability

Unopened vials are stable until the given expiry date when stored under conditions indicated on the vial or kit label. Once reconstituted, the reagent is stable for 8 hours when kept at $^{+}2$ – $^{+}8$°C. The lyophilised product should appear as a dry, straw coloured plug or pieces. Any deviation from this appearance may indicate signs of product deterioration.

Sample Collection And Preparation

Plastic or siliconised glass should be used throughout. Blood (9 parts) should be collected into 3.2% or 3.8% sodium citrate anticoagulant (1 part). Separate plasma after centrifugation at 1500 x g for 15 minutes. Plasma should be kept at $^{+}2$ –$^{+}8$°C or $^{+}18$ – $^{+}24$°C. Testing should be completed within 4 hours of sample collection, or plasma can be stored frozen at -20°C for 2 weeks or -70°C for 6 months. Thaw quickly at $^{+}37$°C prior to testing. Do not keep at $^{+}37$°C for more than 5 minutes[2].

Procedure

A. Manual method

Prepare all reagents as instructed with each pack. Pre-warm both the Dia-PTT reagent to room temperature and Dia-CaCl2 to $^{+}37$°C.

Tube	Calibration Plasma (mL)	Dia-IMIDAZOL (mL)	Activity (%)
1	0,1	0,4	100
2	0,1	0,9	50
3	0,1	1,9	25
4	0,1	3,9	12,5

1. Standard Curve Preparation:
   a. Prepare the above mentioned dilutions in Dia-IMIDAZOL.
   b. Mix without shaking.

2. Patient Sample Preparation:
   a. Prepare a 1+4 dilution of the patient plasma or control plasma in Dia-IMIDAZOL.
   b. Mix without shaking.

3. Testing:
   a. Pipette, in duplicate, 50 µL of factor deficient plasma into a reaction tube.
   b. Add 50 µL of standard, patient or control plasma dilution and incubate at $^{+}37$°C for 2 minutes. **In case of software versions before 2.06 for Coag4D and 2.02 for Coag2D you have to control the indicated incubation time by stopwatch!**
   c. In the case of software versions 2.06 (for Coag4D) and 2.02 (for Coag2D) this step has already been built in the software.
   d. Add 50 µL mL of Dia-PTT reagent and incubate for 5 minutes at $^{+}37$°C.
   e. Add 50 µL of 0.025 M Dia-CaCL2 solution while simultaneously starting a stopwatch.
   f. Determine the clot time for each of the standard, control or patient dilutions.

g. Plot % Activity (X-axis) versus Mean Clot Time (Y-axis) for the standards on 2 cycle log-log graph paper.
h. A straight line should be obtained.

Interpretation of results

Interpolate patient values from the curve. Calculate exact patient or control values by correcting for differences in calibration plasma values as follows:

Correct Activity (%) =	(Calibration plasma Reference Value / 100)	x	Interpolated Patient or Control Value

B. Automated method

Refer to the appropriate instrument operator manual for detailed instructions or contact Diagon Ltd. for instrument specific application guides.

Limitations

The results obtained with Factor Deficient Plasmas depend on several factors strongly associated with instrumentation, types of reagents, deficient substrates and laboratory to laboratory variations. Each laboratory should establish an expected range for the particular instrument-reagent system.

Quality control

Each laboratory should establish a quality control program. Normal and abnormal control plasmas should be tested prior to each batch of patient samples, to ensure satisfactory instrument and operator performance. If controls do not perform as expected, patient results should be considered invalid. Diagon Ltd. supplies the following controls available for use with this product:

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Cat.No.: 9405 Dia-CONT Spec P

Reference values

Reference values can vary between laboratories depending on the techniques and systems in use. For this reason each laboratory should establish its own normal range. Expected values for factor activity are 50–150%[6].

Performance characteristics

Each laboratory should establish its own performance data. Diagon Ltd. intrinsic factor assays are designed to give a linear standard curve from 10–150%. Within run and between run precisions are expected to be <5%, using a range of automated instruments.

References

1. Penner JA (1979) The University of Michigan Medical School Blood Coagulation Laboratory Manual, 14th Ed., University Publications, Ann Arbor, 72-78.
2. Clinical and Laboratory Standards Institute (2008) Collection, Transport and Processing of Blood Specimensfor Testing plasma-based Coagulation Assays and Molecular Haemostasis Assays: Approved Guideline, 5th edn. CLSI: H21-A5.
3. Kirkwood TBL *et al.* (1977) Identification of Sources of Variationin Factor VIII Assay, *British Journal of Haematology*, **37:**559-568.
4. Goldenfarb MD (1971) Reproducibility in Coagulation Assays, *AJCP*, **55:**561-564.

5. Palkuti HA and Longberry JR (1973) A Precision Study of Coagulation Factor Assay Techniques, *AJCP*,**59:**231-235.

6. Triplett DA, Harms CS (1981) Procedures for the Coagulation Laboratory, Am. Society for Clin. Path, Chicago, 36

DIAGON LTD.
Baross u. 48-52, 1047 Budapest, Hungary
Tel.: +36 1 3696500
Fax.: +36 1 3696301
Web: www.diagon.com
E-mail: diagon@diagon.com

Symbols			
IVD	In vitro diagnostics devices		Check in user manual
	Biohazard		Temperature range
	Manufacturer		Expiry date
LOT	LOT number	CE	CE conformity sign
REF	Catalogue number		