PrecisionBioLogic

CRYO*check*™ IVD

FACTOR DEFICIENT PLASMAS

FACTOR II DEFICIENT PLASMA

Intended Use

CRYO*check* Factor II Deficient Plasma is for clinical laboratory use as a deficient substrate in the quantitative determination of factor II activity in 3.2 % citrated human plasma based on the prothrombin time (PT) assay. It is intended to be used in identifying factor II deficiency and in the management of hypoprothrombinemia. For in vitro diagnostic use.

Summary and Principle

Deficiencies in coagulation factors may have congenital or acquired etiologies and can compromise in vivo hemostasis[1]. Factor II (prothrombin) is a single-chained glycoprotein with a molecular weight of 72 000 Da and is important for both intrinsic and extrinsic coagulation[2]. Plasma samples deficient in coagulation factor II exhibit a prolonged PT and activated partial thromboplastin time (APTT). Factor II deficiency is commonly diagnosed through the use of a modified PT assay. When a patient sample is mixed with factor II deficient plasma, the degree of correction of the PT is proportional to the level of factor II in the patient plasma[3].

Factor II deficiency, also known as hypoprothrombinemia or prothrombin deficiency, is among the rarest of all bleeding disorders, occurring in ~1 case in 1 to 3 million[4]. Hypoprothrombinemia is characterized by constantly low factor II antigen and activity. Patients with <5% prothrombin coagulant activity (factor II activity) are considered severe and may present with prolonged postinjury bleeding, mucosal bleeding, hematomas, and hemarthroses. Factor II activity levels are monitored through treatment and trough levels of 20-30% FII activity are recommended[5,6,7].

Reagents

CRYO*check* Factor II Deficient Plasma consists of normal citrated human plasma, which has been depleted of factor II by immunoadsorption. The plasma is then buffered with HEPES buffer, aliquoted, and rapidly frozen. Factor II has been assayed at less than 1 % of normal levels by both functional and antigenic methods. Other factors have been assayed and results are provided on the Quality Control Certificate that accompanies each lot number.

FOR PRESCRIPTION USE ONLY.

To be used by professional laboratory personnel in manual or automated methods.

Product ingredients: Citrated human plasma, buffered with 0.01 M HEPES.

Storage, Preparation and Handling

When stored at -40 to -80 °C, CRYO*check* Factor II Deficient Plasma is stable to the end of the month indicated on the product packaging.

Thaw each vial at 37 °C (±1 °C) in a waterbath using the waterbath "floatie" thawing device (provided separately). Thawing times are important and should be strictly adhered to. **The use of a dry bath or heating block for thawing is not recommended.** The use of a timer is recommended. Refer to the Thawing Table for recommended thawing times according to aliquot size. Allow thawed plasma to acclimate to room temperature (18 to 25 °C) and invert each reagent gently prior to use.

Thawing Table	
Aliquot Size	**37 °C (±1 °C) Waterbath**
1.0 mL	4 minutes
1.5 mL	5 minutes

When first thawed, CRYO*check* Factor II Deficient Plasma may be used for up to eight hours on board the analyzer (15 to 19 °C) or if capped in the original vial and maintained at 2 to 8 °C. Allow refrigerated plasma to acclimate to room temperature (18 to 25 °C) and invert gently prior to use. **Thawed material should be discarded after eight hours and should not be refrozen.**

Availability

Product	**Catalog #**	**Format**
CRYO*check* Factor II Deficient Plasma	FDP02-10	25 vials x 1.0 mL
	FDP02-15	25 vials x 1.5 mL

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use if using an automated coagulation analyzer to perform the assay.

Procedure

After thawing and preparing CRYO*check* Factor II Deficient Plasma, use in accordance with established laboratory procedures for quantitative assessment of factor II.

Material Provided

- CRYO*check* Factor II Deficient Plasma

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (±1 °C)
- Floatie for thawing vials in waterbath
- Assay reagents (PT reagent)
- Owren-Koller Buffer or equivalent
- Coagulation instrument or assay system (automated method)
- Calibration plasma (e.g. CRYO*check* Normal Reference Plasma)

- Quality control material (e.g. CRYO*check* Reference Control Normal, CRYO*check* Abnormal 1 Reference Control, CRYO*check* Abnormal 2 Reference Control)
- 2-cycle log-log graph paper (manual method)
- Plastic test tubes (e.g. 12 x 75 mm)
- Sample cups (automated method)
- Plastic disposable pipettes
- Volumetric pipette (manual method)
- Timer (manual method)

Standard Curve Preparation

Methods may vary according to instrumentation used. Consult the instrument manufacturer's instruction manual for recommended factor assay (extrinsic) protocols.

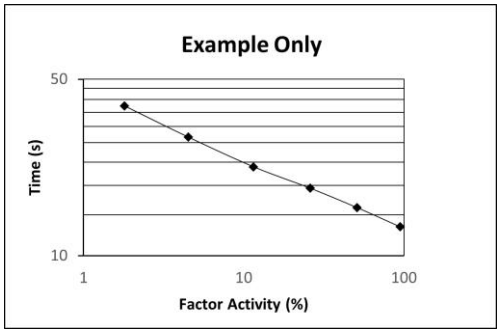
If performing a manual method, prepare a standard curve as follows:

1. Prepare assay reagents, calibration plasma, and buffer according to manufacturer's directions.
2. Make serial dilutions of calibration plasma from 1:10 to 1:320 in buffer as follows:

Tube No.	Volume of Buffer	Volume of Calibration Plasma	Dilution	% Factor
1	1.8 mL	0.2 mL calibration plasma	1:10	100
2	1.0 mL	1.0 mL of Tube No. 1	1:20	50
3	1.0 mL	1.0 mL of Tube No. 2	1:40	25
4	1.0 mL	1.0 mL of Tube No. 3	1:80	12.5
5	1.0 mL	1.0 mL of Tube No. 4	1:160	6.25
6	1.0 mL	1.0 mL of Tube No. 5	1:320	3.12

*Note: This is an **example only** of a serial dilution profile prepared using calibration plasma with a factor II level of 100 %. Always be sure to utilize the lot-specific factor II level of the calibration plasma in use. If using CRYOcheck Normal Reference Plasma, refer to the lot specific Assay Certificate.*

3. Pre-warm thromboplastin to 37 °C (±1 °C).
4. To a coagulation reaction cuvette, add 0.1 mL of CRYO*check* Factor II Deficient Plasma and 0.1 mL of Tube No. 1 (100 % of factor). Mix and incubate according to manufacturer's directions.
5. Add 0.2 mL of pre-warmed thromboplastin and simultaneously initiate the clot timer. Record clotting in seconds.
6. Repeat steps 4 and 5 for Tube Nos. 2 to 6.
7. On log-log graph paper plot clotting times in seconds (y-axis) vs. % of factor II activity (x-axis).
8. Construct the standard curve by drawing the best straight line to fit through the plots.



Specimen Collection and Preparation

Patient samples should be collected into 105 to 109 mmol/L sodium citrate dihydrate anticoagulant (3.2 % w/v) in a ratio of 9 parts blood to 1 part anticoagulant. Patient plasma is derived by centrifugation at 1500 x g for 15 minutes in order to achieve platelet-poor plasma (<10 000 platelets/µL) and should be tested within four hours of collection when maintained at room temperature (15 to 25 °C) in accordance with the Clinical Laboratory Standards Institute (CLSI) guidelines[8]. If samples cannot be tested within four hours, the plasma sample can be stored frozen ≤ -70 °C for up to 24 months.

Assay Procedure

Manual Method:

1. Prepare a 1:10 dilution of patient plasma with buffer.
2. Repeat steps 3 through 5 of Standard Curve Preparation, substituting diluted patient plasma for diluted calibration plasma.
3. Read the percent factor II activity from the standard curve by finding the point where the clotting time intercepts the curve, then reading the percent factor II activity off the x-axis.
4. Further dilutions of patient plasma may be prepared and tested to confirm the value.

Automated Method:

1. Prepare CRYO*check* Factor II Deficient Plasma according to Storage, Preparation and Handling instructions above.
2. Prepare one vial per 14 tests or pool two vials when generating a calibration curve.
3. Prepare instrument according to the manufacturer's instructions for use.
4. Prepare assay reagents (e.g. PT reagent, calibration plasma, buffer) according to manufacturer's instructions for use and load on the instrument.
5. Load the thawed CRYO*check* Factor II Deficient Plasma vial(s) onto the instrument.
6. Load samples on the instrument.
7. Measure the FII activity of plasma samples using the appropriate instrument protocol.

Results and Interpretation

Factor II activity values recovered below the normal range may be indicative of a factor II deficiency (congenital or acquired). Each laboratory should establish its own normal range for factor II activity in accordance with CLSI guidelines[9].

Quality Control

Each laboratory should establish its own quality control (QC) ranges, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own confidence level established in the laboratory. These QC ranges may then be used to monitor and validate the integrity of the testing system[10]. For all coagulation tests, the laboratory must include at least two levels of control for every eight hours of operation and any time a change in reagents occurs[11].

Limitations of Procedure

When proper control values are not obtained, assessment of each component of the test system including reagents, control plasmas, instrumentation and operator technique must be undertaken in order to ascertain that all other components are functioning properly.

Expected Values

Expected values may vary according to reagent, instrument and technique employed as well as population age and characteristics. It is recommended each laboratory establish its own normal range for factor II activity.

A reference interval study was conducted in accordance with CLSI EP28-A3c[12] using three lots of CRYO*check* Factor II Deficient Plasma on the Stago Compact Max and Stago Evolution, using RecombiPlasTin 2G, or NeoPlastine CI Plus. Plasma samples from 307 normal, ostensibly healthy individuals were tested. The reference interval was established by calculating the non-parametric 95 % confidence interval of the combined data and was determined to be 71 to 140 % FII activity.

Performance Characteristics

Refer to the Quality Control Certificate for clotting factor specifications with each lot number of CRYO*check* Factor II Deficient Plasma. When used according to recommended methods, results are subject to the limitations of the assay system (i.e. reagents, instrument) in use.

Method Comparison

Accuracy of factor II activity measurement when CRYO*check* Factor II Deficient Plasma was used in a modified PT assay (using STA Neoplastine CI Plus 10, on a Stago STA-R Evolution) was assessed through a method comparison study where the recovery of factor II activity for 44 samples was compared to a comparator product Stago FII Def. The results were analyzed according to CLSI EP09c[13] by weighted Deming regression analysis. Regression statistics show that CRYO*check* Factor II Deficient Plasma performed equivalently to the comparator method.

N	Slope		Intercept		Pearson Correlation Coefficient (R)
	Value	95 % CI	Value	95 % CI	
44	0.983	0.949, 1.00	-0.132	-1.02, 2.20	0.990 (r^2 = 0.979)

Absolute predicted biases at medical decision levels are reported below.

Medical Decision Level (FII %)	Predicted Absolute Bias (%)	Lower CI (%)	Upper CI (%)
5	-0.219	-1.04	1.98
10	-0.306	-1.08	1.76
50	-0.999	-1.75	-0.121
100	-1.87	-3.48	-0.701
150	-2.73	-5.93	-0.735

Precision

The precision of one lot of CRYO*check* Factor II Deficient Plasma when used with a PT reagent on a coagulation analyzer to quantify factor II activity in two levels of controls, CRYO*check* Reference Control Normal (RCN) and CRYO*check* Abnormal 1 Reference Plasma (ARP1), was assessed by analyzing these controls over 20 days in duplicate, for two runs per day using multiple operators. The resulting data were analyzed as per CLSI EP05-A3[14] to calculate within-run precision (repeatability), between-run precision, between-day precision, and within-lot precision.

The expected precision estimates using a single representative lot of CRYO*check* Factor II Deficient Plasma are displayed below. The results demonstrated within-lot precision of <10 % CV for all.

HemosIL RecombiPlasTin 2G on the STA-R Evolution									
Sample	Mean Value (%)	Within-Run		Between-Run		Between-Day		Within-Lot	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
RCN	101	2.1	2.1	1.2	1.2	1.2	1.2	2.7	2.7
ARP1	39	0.7	1.8	0.3	0.8	0.6	1.6	1.0	2.5

Linearity

A linearity study was conducted in accordance with CLSI EP06-Ed2[15] using three lots of CRYO*check* Factor II Deficient Plasma. Plasma with high factor II activity (>200 %) was combined with factor II deficient plasma (<1 %) to create nine sample dilutions with estimated factor II activity in the range of 0 to 239 %. The results support a linear range of 12 to 155 %.

Interferences

Interference studies were conducted according to CLSI EP07[16] using a single lot of CRYO*check* Factor II Deficient Plasma in a modified PT assay. Plasma samples were spiked with possible interferents, and 10 replicates were tested alongside 10 replicates of the corresponding blank matrix control. The following substances showed no interference up to the concentrations indicated.

Substance Tested	Test Concentration
Hemoglobin	≤500 mg/dL
Intralipid	≤1000 mg/dL
Bilirubin (unconjugated)	30 mg/dL
Lupus Anticoagulant	≤1.8 dRVVT ratio

Refer to specific PT reagent instructions for more details on interference claims. Certain anticoagulants, such as warfarin, heparin, direct thrombin and FXa inhibitors are known interferents. The presence of nonspecific factor inhibitors is also known to interfere with one-stage clotting assays[9].

Precautions/Warnings

Do not use the product if it is thawed upon receipt, if it has been stored outside the recommended storage conditions, if the vials appear cracked, or if upon thawing the product appears to have clotted. Transferring the material into another container other than siliconized glass or polypropylene could have a performance impact and is not recommended.

Any serious incident that has occurred in relation to the use of this device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or patient is established.

A Summary of the Safety and Performance of this device can be found on the EUDAMED database.

All blood products should be treated as potentially infectious. Source material from which this product was derived was found to be negative when tested in accordance with current required tests for transfusion-transmitted diseases. No known test method can offer complete assurance that components derived from human blood will not transmit infectious agents. Accordingly, these human blood-based products should be handled and discarded as recommended by local regulations for any potentially infectious human specimen[17].

Bibliography

1. Biggs R. Human blood coagulation, haemostasis and thrombosis, 3rd ed. Oxford: Blackwell Scientific Publications; 1984.
2. Halkier T. Mechanisms in blood coagulation, fibrinolysis, and the complement system. Cambridge: Cambridge University Press; 1991.
3. Triplett DA, Smith C. Routine testing in the coagulation laboratory. In: Triplett DA, editor. Laboratory evaluation of coagulation. Illinois: ASCP Press; 1982. p. 28-51.
4. Peyvandi F, Menegatti M. Treatment of rare factor deficiencies in 2016. *Hematology Am Soc Hemato Educ Program.* 2016 Dec; 2016(1):663-9.
5. Menegatti M, Peyvandi F. Treatment of rare factor deficiencies other than hemophilia. *Blood.* 2019 Jan; 133(5):415-424.
6. Schwartz, RA. Factor II Deficiency: Practice Essentials, Background, Pathophysiology. *Medscape.* 18 March 2024, https://emedicine.medscape.com/article/209742-overview.
7. Peyvandi F, Palla R, Menegatti M, *et al*. European Network of Rare Bleeding Disorders Group. Coagulation factor activity and clinical bleeding severity in rare bleeding disorders: results from the European Network of Rare Bleeding Disorders. *J Thromb Haemost.* 2012; 10(4):615-621.
8. CLSI. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays, 6th ed. CLSI guideline H21. Clinical and Laboratory Standards Institute; 2024.
9. CLSI. Determination of Coagulation Factor Activities Using the One-Stage Clotting Assay, 2nd ed. CLSI guideline H48. Clinical and Laboratory Standards Institute; 2016.
10. Cembrowski GS, Carey RN. Laboratory Quality Management. Chicago: ASCP Press; 1989. p. 166-171.
11. CLIA 2004 – Code of Federal Regulations, 42CFR493.1269; 2004.
12. CLSI. Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory, 3rd ed. CLSI guideline EP28-A3c. Clinical and Laboratory Standards Institute; 2010.
13. CLSI. Measurement Procedure Comparison and Bias Estimation Using Patient Samples, 3rd ed. CLSI guideline EP09c. Clinical and Laboratory Standards Institute; 2018.
14. CLSI. Evaluation of Precision of Quantitative Measurement Procedures, 3rd ed. CLSI guideline EP05-A3. Clinical and Laboratory Standards Institute; 2014.
15. CLSI. Evaluation of the Linearity of Quantitative Measurement Procedures, 2nd ed. CLSI guideline EP06. Clinical and Laboratory Standards Institute; 2020.
16. CLSI. Interference Testing in Clinical Chemistry, 3rd ed. CLSI guideline EP07. Clinical and Laboratory Standards Institute; 2018.
17. Biosafety in microbiological and biomedical laboratories, 6th ed. Centers for Disease Control and Prevention/National Institutes of Health; 2020.

Symbols Used

IVD	In vitro diagnostic medical device		Manufacturer
LOT	Batch code	EC REP	Authorized representative in the European Community / European Union
REF	Catalogue number	CH REP	Authorized representative in Switzerland
	Use by date	Rx Only	For prescription use only
	Temperature limit		Consult electronic instructions for use
	Biological risks		

CE 0123

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe—Westervoortsedijk 60, 6827 AT Arnhem, The Netherlands

CH REP Swiss Authorized Representative (Regulatory affairs only)
Endotell AG—Gewerbestrasse 25, 4123 Allschwil, Switzerland

Precision BioLogic Inc.
140 Eileen Stubbs Avenue | Dartmouth, Nova Scotia | B3B 0A9 | Canada

Tel: 1.800.267.2796 / +1.902.468.6422
Fax: 1.800.267.0796 / +1.902.468.6421

www.precisionbiologic.com