PrecisionBioLogic

CRYO*check*™ IVD

FACTOR DEFICIENT PLASMAS

FACTOR II DEFICIENT PLASMA

Intended Use

CRYO*check* Factor II Deficient Plasma is recommended for use as a deficient substrate in clot-based factor II assays using the one-stage prothrombin time (PT).

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].

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.

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 methods can offer assurance that products derived from human blood will not transmit infectious agents. Accordingly, these human blood-based products should be handled and discarded as recommended for any potentially infectious human specimen[4].

Storage 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. **The use of a dry bath or heating block for thawing is not recommended**. Thawing times are important and should be strictly adhered to. The use of a timer is

recommended. Refer to the Thawing Table for recommended thawing times based on aliquot size. Allow thawed plasma to acclimate to room temperature (18 to 25 °C) and invert gently prior to use.

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

CRYO*check* Factor II Deficient Plasma may be used for up to eight hours after thawing, 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
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.

Procedure

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

Materials Provided

- CRYO*check* Factor II Deficient Plasma

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Assay reagents
- $CaCl_2$
- Owren-Koller Buffer or equivalent
- Coagulation instrument or assay system
- 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
- Plastic test tubes (e.g. 12 x 75 mm)
- Sample cups
- Plastic disposable pipettes
- Volumetric pipette
- Timer

Standard Curve Preparation

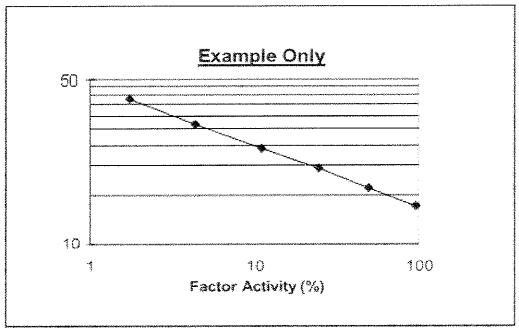
Methods may vary according to instrumentation used. consult the instrument manufacturer's instruction manual for recommended factor assay (extrinsic) protocols.

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 utilizethe 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 time 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 fit through the plots



Specimen Collection and Preparation

Patient samples should be collected into 105 - 109 mmol/L sodium citrate dihydrate anticoagulant (3.2%) in a ratio of 9 parts blood to 1 part anticoagulant. Patient plasma is derived by centrifugation at 1500 x g for 15 minutes and should be tested within four hours of collection when maintained at 2 to 4 °C in accordance with CLSI guidelines[5].

Assay Procedure

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.

Results

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[6].

Quality Control

Each laboratory should establish its own quality control (QC) ranges using acceptable statistical methods. These QC ranges may then be used to monitor and validate the integrity of the test system[7]. 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[8].

Limitations of the 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. It is recommended each laboratory establish its own normal range for factor II 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.

Bibliography

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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. Biosafety in Microbiological and Biomedical Laboratories 6th ed. Centers for Disease Control and Prevention / National Institutes of Health, 2020.
5. Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline – Fifth Edition, CLSI, H21-A5; Vol. 28, No. 5, 2008.
6. Determination of Factor Coagulant Activities Using the One-Stage Clotting Assay, CLSI; Approved Guideline – Second Edition, CLSI, H48, 2016.
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8. CLIA 2004 – Code of Federal regulations, 42CFR493.1269, 2004.

Symbols Used

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

EC REP European Authorized Representative (Regulatory affairs only)
Emergo Europe—Prinsessegracht 20, 2514 AP The Hague, The Netherlands

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