

CRYOcheck™ **IVD**

FACTOR DEFICIENT PLASMAS

PREKALLIKREIN DEFICIENT PLASMA

Intended Use

CRYOcheck Prekallikrein Deficient Plasma is recommended for use as a deficient substrate in clot-based prekallikrein assays using the activated partial thromboplastin time (APTT).

Summary and Principle

Deficiencies in coagulation factors may have congenital or acquired etiologies and can compromise in vivo hemostasis¹. Prekallikrein (Fletcher factor) is a single-chained glycoprotein with a molecular weight of 85,000 - 88,000 Da and is necessary for intrinsic coagulation². Plasma samples deficient in prekallikrein exhibit a prolonged APTT which shortens to normal on prolonged incubation with an activating reagent. Prekallikrein deficiency is commonly diagnosed through the use of a modified APTT assay. When a patient sample is mixed with prekallikrein deficient plasma, the degree of correction of the APTT is proportional to the level of prekallikrein in the patient plasma^{3,4}:

Reagents

CRYOcheck Prekallikrein Deficient Plasma consists of citrated human plasma from a single prekallikrein deficient donor, who has been assayed at less than 1% of normal levels for prekallikrein. The plasma is then buffered with HEPES buffer, aliquoted, and rapidly frozen. Other factors have been assayed and results are provided on the certificate of Analysis 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 specimen⁵.

Storage and Handling

When stored at -40 to -80°C, CRYOcheck Prekallikrein 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

CRYOcheck Prekallikrein 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
Prekallikrein Deficient Plasma	FDPK-10	10 vials x 1.0 mL

Instruments

Each lab should prepare the local instrument in accordance with the manufacturer's instructions for use.

Procedure

After thawing and preparing CRYOcheck Prekallikrein Deficient Plasma, use in accordance with established laboratory procedures for the quantitative assessment of Prekallikrein.

Materials Provided

- CRYOcheck Prekallikrein Deficient Plasma

Materials Required but not Provided

- Waterbath capable of maintaining 37 °C (± 1 °C)
- Assay reagents
- CaCl₂
- Owren's Koller Buffer or equivalent
- Coagulation instrument or assay system
- Calibration plasma (e.g. CRYOcheck Normal Reference Plasma)
- Quality control material (e.g. CRYOcheck Reference Control Normal, CRYOcheck Abnormal 1 Reference Control)
- 2 cycle log-log graph paper
- Plastic test tubes (e.g. 12 x 75 mm)
- Reaction Cuvette
- Plastic disposable pipettes
- Volumetric pipette
- Timer

Standard Curve Preparation

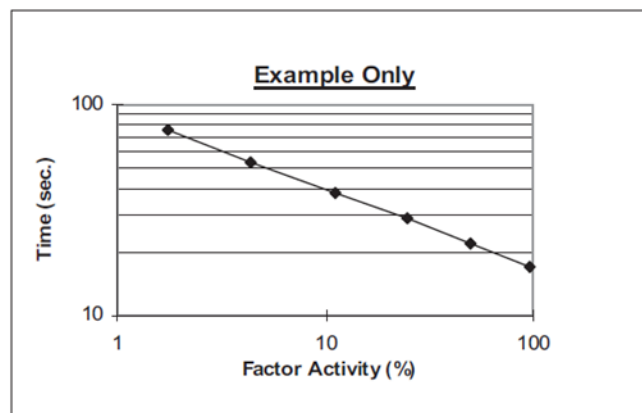
Methods may vary according to instrumentation used. consult the instrument manufacturer's instruction manual for recommended factor assay (intrinsic) protocols.

1. Prepare assay reagents, calibration plasma, and buffer according to manufacturer's directions.
2. Make serial dilutions of calibration plasma from 1:160 to 1:5120 in buffer as follows:

Tube No.	Volume of Buffer	Volume of Calibration Plasma	Dilution	% Factor
1	1590 μ L	10 μ L calibration plasma	1:160	100
2	1.0 mL	1.0 mL of tube no. 1	1:320	50
3	1.0 mL	1.0 mL of tube no. 2	1:640	25
4	1.0 mL	1.0 mL of tube no. 3	1:1280	12.5
5	1.0 mL	1.0 mL of tube no. 4	1:2560	6.25
6	1.0 mL	1.0 mL of tube no. 5	1:5120	3.12

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

3. Prewarm APTT reagent and calcium chloride to 37 °C (\pm 1 °C).
4. To a coagulation reaction cuvette, add 0.1 mL of cryocheck Prekallikrein Deficient Plasma, 0.1 mL of Tube No. 1 (100% of factor), and 0.1 mL of prewarmed APTT reagent. Mix and incubate according to the manufacturer's directions.
5. Add 0.1 mL of prewarmed calcium chloride and simultaneously initiate the clot timer. Record clotting times 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 prekallikrein 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 guidelines⁶.

Assay Procedure

1. Prepare a 1:160 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 prekallikrein activity from the standard curve by finding the point where the clotting time intercepts the curve, then reading the percent prekallikrein activity off the x-axis.
4. Further dilutions of patient plasma may be prepared and tested to confirm the value.

Results

Prekallikrein activity values recovered below the normal range may be indicative of a prekallikrein deficiency (congenital or acquired). Each laboratory should establish its own normal range for prekallikrein activity in accordance with CLSI guidelines⁷.

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 system⁸. 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 occurs⁹.

Limitations of the Procedure

Plasmas deficient in factor XII or containing lupus anticoagulants may give falsely low prekallikrein results. 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 prekallikrein activity.











Performance Characteristics

Refer to the Quality Control Certificate for clotting factor specifications with each lot number of *CRYOcheck* Prekallikrein 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

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. Hathaway WE, Belhasen LP, Hathaway HS. Evidence of a new thromboplastin factor: case report, coagulation studies and physiochemical studies. *Blood* 1965; 26(5):521-32.
5. Biosafety in Microbiological and Biomedical Laboratories 6th ed. Centers for Disease Control and Prevention / National Institutes of Health, 2020.
6. 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.
7. Determination of Factor Coagulant Activities Using the One-Stage Clotting Assay; Approved Guideline – Second Edition, CLSI, H48, 2016.
8. Cembrowski GS, Carey RN. Laboratory quality management. Chicago: ASCP Press; 1989. p. 166-171.
9. CLIA 2004 – Code of Federal regulations, 42CFR493.1269, 2004

Symbols Used

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



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



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