Factor X Deficient Plasma (Cat. No K-741)

For use in the quantitative determination of coagulation factors in plasma.

For in vitro diagnostic use. Store at 2° - 8°C

I. Intended Use
Factor Deficient Plasmas are intended for use in the quantitative determination of coagulation factors in plasma, using manual mechanical or photo optical methods of clot detection.

II. Summary and Principles
Factor activity in plasma is assayed by the amount of Prothrombin Time (PT) or Activated Partial Thromboplastin Time (APTT) correction produced by the test plasma when mixed with factor deficient plasma. The correction of the unknown is compared to that produced by a reference plasma of known normal factor activity, such as Reference Plasma.

III. Reagents
For in vitro diagnostic use.
Composition:
Factor Deficient Plasmas: Derived from congenitally deficient donors collected with sodium citrate anticoagulant, except Factor II. Factor II deficient is a mixture of human serum and bovine plasma. Buffer is added prior to lyophilization. All plasmas contain <1% of the deficient factor.
CAUTION! POTENTIAL BIOHAZARD: Contains human source material. While each human serum or plasma donor unit used in the manufacture of this product was tested by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2, all products manufactured using human source material should be handled as potentially infectious. Because no test method can offer complete assurance that hepatitis B or C viruses, HIV, or other infectious agents are absent, these products should be handled according to established good laboratory practices.
Store unopened vials at 2-8°C. Reconstitute with 1.0 mL of distilled water. Swirl gently until solution is complete. The reconstituted plasmas are stable for 1 hour at 2-8°C. Gently mix contents prior to each use.
Diluting Fluid (BBS): Barbital buffer in saline with sodium azide as a preservative.
Warning: BBS contains 2mM sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Dilute with running water before discarding, and then flush with large volumes of water. These precautions are recommended to avoid deposits in metal piping in which explosive conditions may develop.
Erratic values, product color variations, or lack of vacuum in the vials (plasmas only) could indicate product deterioration. However, poor performance could also be due to other factors within the test system.

IV. Specimen Collection:
3.2% (0.109M) trisodium citrate anticoagulant is recommended for coagulation assays. Avoid hemolysis and contamination by tissue fluids. Samples that have less than 90% of the expected fill volume should be rejected. Centrifuge blood for 15 minutes at 2500 x g. Test within 2 hours if samples are held at 22-24°C. For more details on specimen collection and storage, see NCCLS Document H21-A31.

V. Test Procedure
This product is suitable for use with manual, mechanical, photo-optical or nephelometric methods for clot detection. Consult instreument manufacturer for more specific guidelines. The following procedural outline assumes use of a manual method.
Materials Provided (Kit contains one of the following): Factor Deficient Plasmas.

Materials Required, But Not Provided:
PT and/or APTT Reagent
Pipets
Reference Plasma (Calibration plasma)
Distilled water
12 x 75 mm plastic test tubes

Notes on Procedure:
1. Perform all testing in duplicate.
2. Prepare dilutions immediately prior to testing.
3. A new standard curve must be prepared each time testing is performed.

Preparation of Factor Activity Reference Curve:
1. Reconstitute one vial of Reference Plasma. See package insert accompanying this product for reconstitution instructions, storage and stability.
2. Reconstitute and pool sufficient vials of the Factor Deficient Plasma to allow 0.2 mL for each dilution to be tested.
3. Label 4 plastic test tubes and pipet Diluting Fluid and Reference Plasma according to the chart below. Cap and mix gently by inversion.

Tube#	Diluting Fluid	Reference Plasma	Dilution	Dilution Factor	Factor activity in dilution*
1	0.9 ml	0.1 ml	1:10	1	110
2	1.9 ml	0.1 ml	1:20	2	55
3	3.9 ml	0.1 ml	1:40	4	27.5
4	3.95 ml	0.05 ml	1:80	8	13.8
5	1.0 ml	1.0 ml Tube#4	1:160	16	6.9

*Chart is for example only. Based on assigned value of 110% for Reference Plasma factor activity.

Test Standards, Controls and Test Samples:
Prepare dilutions of patient and control samples. At least three different dilutions of the patient samples should be tested, beginning with the same dilution as the first point on the curve (1:10 in this case).

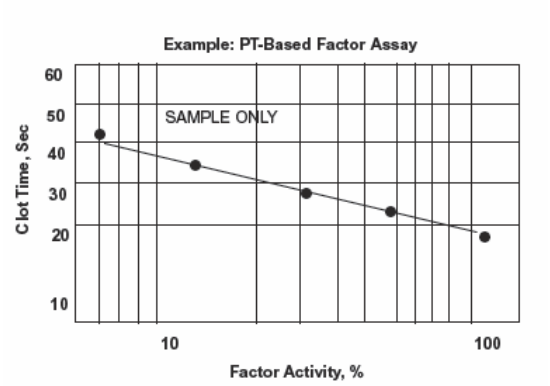
PT-based factors II, V, VII, and X:
1. Prewarm reconstituted Thromboplastin reagent to 37°C.
2. Add 0.1 mL Factor Deficient Plasma and 0.1 mL of standard, patient, or control plasma dilution to a cuvette. Warm to 37°C for 3-5 minutes.
3. Add 0.2 mL reagent and time clot formation.

APTT-based factors VIII, IX, XI, and XII:
1. Prewarm Calcium Chloride reagent to 37°C. See package insert with APTT reagent for information on the appropriate Calcium Chloride to use.
2. Pipet 0.1 mL activator into a cuvette.
Incubate at least 3 minutes, but no more than 20 minutes before proceeding.
3. Add 0.1 mL Factor Deficient Plasma and 0.1 mL of standard, patient or control plasma dilution to the cuvette. Mix well. Incubate for exactly 3 minutes when using APTT-EA and for exactly 5 minutes when using APTT-P.
4. Add 0.1 mL prewarmed Calcium Chloride and time clot formation.

VI. Results

Reference Curve:

1. For each dilution of the Reference Plasma, calculate the mean of duplicate clotting times to the nearest 0.1 second.
2. Calculate the factor activity in each dilution of the Reference Plasma. The first dilution (1:10 in the example) corresponds to 100% factor activity, therefore the factor activity of this dilution is equal to the assay value of the Reference Plasma. The dilution factor represents the relationship between the first dilution and subsequent dilutions. Divide the assay value of the standard by the dilution factor to determine the percent activity for each dilution. Refer to the table in section V.
3. Plot the mean clotting time of each dilution against its percent activity using log-log graph paper. Construct a straight line of best fit.



Test Specimens and Controls:

1. For each dilution, calculate the mean of duplicate clotting times to the nearest 0.1 second.
2. Locate the mean clotting time on the vertical axis of the reference curve. Find the corresponding point on the reference line and read the percent activity on the horizontal axis of the graph. Clotting times outside the curve boundaries should not be used.
3. Multiply the percent activity from the graph by the appropriate dilution factor to determine the actual percent activity for each dilution. If the values for the individual dilutions do not check with each other, new dilutions should be made and the testing repeated. If they still do not agree, you should consider the possibility of an inhibitor.

VII. Quality Control

Assayed reference plasmas such as Abnormal Coagulation Reference Plasma and an additional lot of Reference Plasma should be tested to validate the reference curve. Abnormal Coagulation Reference Plasma is assigned values for coagulation factors in the abnormal range. Each lot will vary, but the values are expected to be approximately 30-50% of normal[3]. This control plasma provides an excellent quality control check on the accuracy of factor determinations at the low end of the reference curve. Testing an additional lot of Reference Plasma (other than the one used for the reference curve) is an ideal control in the normal range. Actual values recovered depend on individual technique, instrument, standard, and reagent used.

VIII. Limitations

Variables such as the standard used, temperature, reagent stability, plasma sample conditions, instrument performance, and individual technique can influence final results. Always follow instrument and reagent manufacturers guidelines.

IX. Expected Values

Normal plasma will yield factor activity values between 50-150% of normal.[2]

X. Performance Characteristics

Factor Deficient plasmas and Diluting Fluid will perform according to the limitations of the procedure described herein when tested with Pacific Hemostasis reagents.

XI. References

1. NCCLS: Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays. 3rd edition. Approved guideline. NCCLS Document H21-A3. Wayne, PA, 1998.
2. Powers, L.W. Technical Hematology. In: Diagnostic Hematology. 1989. Bircher S. (Ed.). The C.V. Mosby Co., St. Louis, MO. p484.

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