

NoFACT II_i

Immunodepleted Factor II Deficient Substrate Plasma



The **NoFACT II_i** Deficient Substrate Plasma is intended for the quantitative determination of Factor II in patients suspected of having a congenital or acquired deficiency of this coagulation protein.

SUMMARY

Numerous coagulation factors in human blood are required for normal blood clotting. A deficiency of one or more of the factors may result in a clinically significant hemorrhagic condition, the severity of which is proportional to the degree of the deficiency. Factor deficiencies may be congenital or acquired. The congenital deficiencies are usually single deficiency states while the acquired deficiencies may be multiple in nature.

Congenital Factor II deficiency is very rare, however, acquired Factor II deficiency may occur in conjunction with vitamin K deficiency, liver disease, or oral anticoagulant therapy. Rare Factor II deficiencies are seen with lupus anticoagulants.

The quantitative assay for Factor II uses a modification of the prothrombin time (PT) test, and a Factor II deficient substrate plasma.

PRINCIPLE

Factor II, more commonly called prothrombin, is the active precursor of thrombin. Prothrombin is activated to thrombin by a combination of Factor Xa, Factor Va, phospholipid and calcium ions. Severe Factor II deficiency is associated with a prolonged prothrombin time.

A dilution of the test plasma is mixed with an equal volume of Factor II deficient plasma and the clotting time of the mixture determined. By comparing the degree of correction provided by the test plasma with the correction obtained with an acceptable reference standard, the percent activity of Factor II may be determined.

REAGENTS

For In-Vitro Diagnostic Use Only.

Factor II Deficient Substrate Plasma

Ingredients: The reagent is human plasma, which has been immunodepleted using immobilised goat anti-human FII antibody to contain less than 1% Factor II activity. The plasma has been buffered and lyophilized to maximize stability.

WARNING: Potential Biohazard: The **NoFACT II_i** Deficient Substrate Plasma has been found negative when tested for Hepatitis B Antigen (HBsAg) and antibodies to HCV and HIV by FDA licensed tests; however, the deficient plasma should be handled with the same precautions as those observed when handling patient plasmas.

Preparation for Use: Reconstitute each vial of **NoFACT II_i** Deficient Substrate Plasma with 1.0 mL distilled water. Swirl gently, do not shake and allow to stand for 20 minutes at room temperature to insure complete dissolution before use.

Storage and Stability: The lyophilized product is stable until the expiration date printed on the vial when stored at 2 to 8°C. The reconstituted product is stable for 8 hours when stored at 2 to 8°C. After reconstitution, the product should be kept on ice for the duration of testing.

TECHNIQUES

Factor II assays using **NoFACT II_i** Deficient Substrate Plasma can be performed by accepted manual methods or by using optical or electro-mechanical coagulation analyzers.

SPECIMEN COLLECTION AND PREPARATION

Specimen: Plasma obtained from whole blood anticoagulated with 0.1M sodium citrate.

Specimen Collection: Nine parts freshly collected whole blood should be immediately added to one part anticoagulant.

Specimen Preparation: Place the specimen on ice immediately after drawing and centrifuge as soon as possible. To insure platelet-poor plasma, centrifuge the specimen at 2500 x g for 15 minutes (NCCLS H21-A2, 1991). A refrigerated centrifuge is recommended. After centrifugation, the plasma should be removed immediately, using plastic pipettes only.

Storage and Stability: Before and during testing, keep the specimen on ice to insure stability of the factors being tested. If the specimen cannot be tested within 2 hours, quick-freeze the plasma at -70°C.

TEST PROCEDURE

Materials Provided:

10 vials **NoFACT II_i** - 1 mL, 100 determinations

Other Supplies Available from R² Diagnostics

PlasmaRef ARN

PlasmaRef ARL-1

PlasmaRef ARL-2

PHOSPHOPLASTIN RL

Materials required but not provided:

Owren's Veronal buffer

12 x 75 mm plastic test tubes

Stopwatch

Log-log graph paper

Plastic serological pipettes

General Comments

1. Assay patient samples as soon after collection as possible.
2. Sample dilutions must be assayed within 30 minutes after preparation and maintained on ice until tested.
3. Sample dilutions exceeding 1:40 and serial dilutions are not recommended.
4. Run all four of the recommended dilutions on plasma samples to avoid potentially erroneous results due to dilution errors.
5. When performing factor assays, more than one vial of reagent may be needed. To eliminate vial-to-vial variation multiple vials should be reconstituted, allowed to dissolve and pooled, before use.
6. Prepare a new standard curve each time assays are performed. Even though the same lot of reagents may be used, vial-to-vial variation, technique differences and instrument variability require this procedure. PlasmaRef ARN is recommended for use in the preparation of the standard curve.

STEP-BY-STEP METHOD

A. Specimen and Reagent Preparation

1. Centrifuge the blood specimen for 15 minutes at 2500 x g using a refrigerated centrifuge. Remove plasma from the red blood cells immediately and place in a plastic test tube. Keep the plasma on ice for the duration of testing.
2. Reconstitute the appropriate number of vials of **NoFACT II_i** Deficient Substrate with 1.0 mL distilled water. Swirl gently, do not shake and allow to stand approximately 20 minutes at room temperature to insure complete dissolution. Approximately 0.8 mL is required for each specimen assayed.
3. Reconstitute one vial of **PlasmaRef ARN** with 1.0 mL distilled water. Swirl gently, do not shake and allow to stand for 20 minutes to insure complete dissolution.
4. Prepare PT reagent according to the package insert. Pre-warm the reagent to 37°C in a water bath, or temperature controlled instrument reagent well.
5. Number a set of four 12 x 75 mm plastic test tubes for the standard curve and each test specimen.

B. Standard Curve Preparation

1. Prepare the following dilutions of **PlasmaRef ARN**, or fresh normal pool plasma with Owren's Veronal buffer.

Tube	Dilution Ratio	mL Standard	mL Buffer	Defined % Activity
1	1:5	0.1	0.4	100
2	1:10	0.1	0.9	50
3	1:20	0.1	1.9	25
4	1:40	0.1	3.9	12.5

2. Mix tubes gently but adequately. Avoid shaking and excess bubble formation.

3. Perform duplicate prothrombin time tests on each of the standard dilutions as follows.

Pipette into the reaction cup in the order specified:
 100 µL **NOFACT II/** Deficient Substrate
 100 µL 1:5 dilution of **PlasmaRef ARN**

4. Start a stopwatch immediately and incubate the mixture according to the PT reagent package insert.
5. At the end of the incubation period, remix the solution and add 200 µL prewarmed PT reagent and simultaneously start a timer.
6. Record the times required for clot formation.
7. Repeat Steps 3-6 for each dilution in duplicate.

C. Testing of Unknown Specimen

1. Prepare dilutions of each unknown patient specimen as outlined in Step 1 above. Use the same volumes required for the standard.
2. Determine the clotting times of each specimen according to Steps 2-7.

Quality Control

Quality Control for factor assays involves multiple components. Prothrombin Time reagents should be checked for their sensitivity to individual factor deficiencies.

A normal reference control plasma such as **PlasmaRef ARL-1** and an abnormal reference control plasma such as **PlasmaRef ARL-2** can be used to verify instrument and reagent performance.

Careful attention should be given to other test variables. These include; pipettes, calcium chloride, distilled water, timing devices, and buffers.

RESULTS

Standard Curve

1. Use standard log-log paper to plot the curve.
2. Use the X axis (abscissa) to represent percent activity and the Y axis (ordinate) to represent the clotting time in seconds.
3. Determine the average clotting time for each dilution of the standard curve.
4. Plot the points where the line corresponding to the clotting times and the line corresponding to the known percent activity intersect.

EXAMPLE:	Dilution	% Activity	Clotting Time/Sec
	1:5	100	15.0
	1:10	50	17.5
	1:20	25	21.0
	1:40	12.5	25.0

5. Draw a line connecting all four points. This should be a straight line. The lines produced by plotting the clotting times of the standard dilutions and the patient dilutions should be parallel. If the two lines are not parallel, this could indicate the presence of an inhibitor in the patient plasma.

Calculation of Test Results

1. Calculate the mean clotting time for each of the test dilutions.
2. Plot the mean clotting time for each dilution and determine the percent activity.
3. Multiply the percent activity by the dilution factor.
4. Average the four percent activities calculated.
5. To calculate the actual percent factor activity of the mean of the unknown sample, multiply the percent activity of the mean of the sample by the activity of the reference standard (as provided by the manufacturer) used to prepare the curve.

EXAMPLE:

$$\text{Percent Activity of Unknown} \times \text{Defined Activity of Standard} = \text{Actual Percent Factor Activity of Unknown}$$

If % activity of the unknown is 71.2 and the reference plasma assay value is 95% (or 0.95), the calculations would be:

$$71.2 \times 0.95 = 67.6\%$$

When using an un-assayed fresh normal plasma pool to prepare the standard curve, the percent activity is always assumed to be 100% (or 1.0). Fresh normal plasma pools should consist of at least ten healthy donors.

INTERPRETATION OF RESULTS

Factor II deficiency may be either congenital or acquired. Congenital Factor II deficiency affects both sexes, and is extremely rare. The most common causes of acquired Factor II deficiency are liver disease, oral anticoagulant therapy and vitamin K deficiency. Rare Factor II deficiencies are seen with lupus anticoagulants and are associated with significant bleeding risk.

LIMITATIONS

1. The **NoFACT II/** Deficient Substrate Plasma is limited to Factor II activity determinations based on a modified prothrombin test system.
2. Specific variations in the test protocols may be required for use in automated coagulation instruments. The manufacturers recommendations should be followed carefully, especially when any recommendations may vary from those outlined above.

EXPECTED VALUES

Factor II Expected Values: 50-150% of the normal reference plasma

For best results, each laboratory should determine a reference range for its particular population and instrument reagent system.