INTENDED USE
GHI 131-10, Prothrombin complex reagent is intended for the determination of Prothrombin Complex Activity (Owren’s PT activity) and gives information about the activity of the vitamin K-dependent coagulation factors II, VII and X.

FOR IN VITRO DIAGNOSTIC USE

BACKGROUND AND PRINCIPLE OF METHOD
GHI 131-10, Prothrombin Complex Reagent is suitable for analysis of plasma, citrated blood and capillary blood from patients treated with Vitamin K antagonists as Warfarin and for screening to find defects in the intrinsic pathway. When analyzing PT, sample and reagent are being mixed and clotting time is measured. The final volume of sample is 4.8% (1:21). Ion strength (0.15M), PH (7,3), Calcium ion activity (2 mM) and temperature (37°C) are close to the physiological.

Due to the sample dilution the method is relatively insensitive to variation in FV-activity and also to Heparin. This insensitivity for Heparin is enhanced by using sample dilution Buffer with Polybrene (GHI152).

Owens PT activity is expressed in INR (International Normalisation Ratio) which is the quotient between the coagulation time for sample and normal plasma raised to the method’s ISI-value (International Sensitivity Index) (ref.1).

PT activity was previously expressed in percentage of the normal plasma activity. (ref.2.)

- INR = (Patient PT/Normal PT)ISI
- ISI = Log specific International Sensitivity Index for reagent/instrument system.
- The ISI value for the PT reagent is obtained by calibrating with reference calibrators.

The reagent is suitable both with Calcium ions in the reconstituted reagent (1component reagent method, IX) as well as with Calcium added separately when analyzing (2-component-reagent method, 2X).

The one-component-reagent method, IX is more convenient and is suitable for most instruments but have higher demands for needle wash to avoid contamination. In some cases the two component reagent method, 2X is preferred with the reagent reconstituted only in Diluent and with CaCl2 added to start the analysis. The contamination risk gets lower because the reagent is not activated with CaCl2.

PRODUCT DESCRIPTION
GHI131-10 is a freeze-dried reagent consisting of Thromboplastin from rabbit and plasma fraction of bovine origin. Plasma fraction that is a source to Fibrinogen and coagulation factor V is to a high degree deficient in the coagulation factors II, VII and X.

Package: 10 X 10 mL reagent.

Material needed but not included in the kit:
- GHI155, GHI156-5 CaCl2
- GHI150 Owrens Buffer
- GHI152 Owrens Buffer with Polybrene
- GHI154 Diluent

PRECAUTIONS
Avoid contact with skin and eyes. Wear suitable clothing for protection. The reagent contains a low level of Sodium Azide as preservative and should be disposed of in accordance with national and local regulations. Do not empty into drains.

For more information see the Safety Data Sheet.

RECONSTITUTION
Let the Reagent, Diluent and CaCl2 reach room temperature before reconstitution.

Reconstitution 1-reagent method, 1X
For one vial GHI 131-10, Prothrombincomplex reagent, Add 5ml. diluent (GHI154)
Incubate during 10 minutes; mix in the beginning, in the middle and in the end of the period. The reagent will dissolve into a slightly opaque colourless liquid.
Add 5ml, 50mM CaCl2 (GHI155-5)
Reconstitution 2-reagent method, 2X
For one vial GHI 131-10, Prothrombin complex reagent, Add 5ml. diluent (GHI154)
Incubate during 10 minutes; mix in the beginning, in the middle and in the end of the period. The reagent will dissolve into a slight opaque colourless liquid.

STORAGE CONDITIONS AND STABILITY
Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C. Reconstituted reagent is stable for 7 days at 15-25°C and at 2-8°C.

Reagent intended to be used the same day as reconstitution:
- PT-reagent 1X: let stand for two hours after reconstitution before use
- PT-reagent 2X: let stand for one hour after reconstitution before use

It is important to mix the reagent before use but it is not necessary with continuously stiring when reagent is kept on the instrument.

Important recommendation:
If the reconstituted reagent is supposed to be used over a period of several days it is strongly recommended to dilute the reagent the day prior to use to achieve 7 days stability.

The reason is that the sensitivity (ISI) slightly changes between the day for reconstitution (day 0) and day 1 but after that the ISI is stable for 7 days. (See stability data in the certificate)

It is recommended to reconstitute reagent for 7 days at the time.

SPECIMEN COLLECTION AND STORAGE
It is recommended that specimen collection and storage be carried out in accordance with CLSI instructions H21-A5 (ref 4) 9 parts of freshly drawn venous blood are collected into one (1) part 0,13 M tris-nitrateum that must be mixed immediately and thoroughly. The ratio is critical and insufficient filling and the presence of a clot in a specimen is cause for rejection.

Plasma is obtained by centrifugation of anticoagulated blood during 15 minutes at 2500g within 24 hours. Before centrifugation check that there are no coagel in the sample.

ANALYSIS METHOD
For detailed description regarding the test parameters as well as test-performance reference is made to instrument specific applications. Observe the different applications for plasma, citrate- and capillary blood.

Preliminary for plasma, citrate- and capillary blood

- 100 µl plasma + 600 µl Owens buffer 1:7
- 50 µl capillary blood + 200 µl Owens buffer 1:5
- 50 µl citrated blood (1+9) + 170 µl Owens buffer 1:4,4

Procedure plasma
Analysis with one-component reagent
- 100 µl plasma diluted 1:7, pre-heated to 37°C
- 20µl PT reagent 1X, pre-heated to 37°C
Analysis with two-component reagent
- 100 µl plasma diluted 1:7, pre-heated to 37°C
- 10µl PT reagent 2X, pre-heated to 37°C
- 100 µl 25 mM CaCl2 pre-heated to 37°C

For both methods the following is applicable: mix immediately and let react at 37°C.

Note the coagulation time that is the interval from the last addition until coagulation occurs. The PT-activity is determined by comparing the obtained coagulation time with corresponding calibrators with known contents of PT-activity.

Interpretation of analysis result
Normal range INR 0,92-1,20 (70-130%)
Optimal oral anticoagulation treatment with thrombosis prophylas: INR 2-3 (15-25%)
(ref 3)

CALIBRATION
Calibration according to local routines for Owens PT.
QUALITY CONTROL (Not included in the kit)
For reliable quality control of the performance it is recommended to use normal control plasma (GHI 162, GHI163 and GHI164) and abnormal control plasma OKP (GHI 167B, GHI169 and GHI170) and at a frequency in accordance with good laboratory practise.

SPECIFICITY AND INTERFERENCES (plasma)
The reagent is not affected by Bilirubin up to 0,5 g/L, Triglycerides up to 10 g/L, Hemoglobin up to 10 g/L and Heparin* up to 1IE/mL.

- Means unfractionated Heparin and use of dilution buffer GHI150. When using buffer with Polybrene GHI152 the reagent is not affected by unfractionated Heparin up to 4 IE/mL.

It is recommended that each laboratory should establish its own heparin therapeutic range.

TYPICAL PERFORMANCE
Analysis performed on ACL9000 with plasma with two INR levels. (n=81)

<table>
<thead>
<tr>
<th>Level</th>
<th>CV% within series</th>
<th>CV% between series</th>
<th>CV% Total</th>
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<td>0,3</td>
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REFERENCES