INTENDED USE
MediRox MRX Ownen’s PT, Prothrombin Complex reagent is intended for determination of Prothrombin Complex activity and provides 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
MRX Ownen’s PT, Prothrombin Complex Reagent is suitable for analysis of plasma, citrated blood and capillary blood from patients treated with Vitamin K antagonists such as Warfarin and for screening to find defects in the extrinsic pathway.

When analyzing PT, sample and reagent are being mixed and clotting time is measured. The final volume of sample is 4,8% (PT/ISI2) of the PT value (International Sensitivity Index) (ref.2) and can affect the analysis results and the reagent can be used as normal. But it is recommended to remove the precipitate. ISI and MNPT should be established for each lot of reagent on every individual measuring system according to local practise.

PRODUCT DESCRIPTION
MRX Ownen’s PT is a lyophilized reagent consisting of thromboplastin from rabbit and plasma fraction of bovine origin. The bovine plasma fraction is a source to Fibrinogen and coagulation factor V and is to a high degree deficient in the coagulation factors II, VII and X.

Package:
- GHI131-4: 10 x 4 mL (vial size 22x49mm)
- GHI131-10: 10 x 10 mL (vial size 22x49,5 mm)
- GHI131-10SI: 10 x 10 mL (vial size 30x50 mm)
- GHI131-20: 10 x 20 mL (vial size 27,5x60mm)

Material needed but not included in the kit:
- GHI155, GHI155-2, GHI155-5, GHI155-10 25 mM CaCl2
- MRX150 PT Buffer or GHI150 Ownens Buffer
- MRX152 or PT Buffer with Polybrene or GHI152 Ownens Buffer with Polybrene
- GHI154, GHI154-2, GHI154-10 Diluent or CLSI CLRW type water or equivalent (ref 5)

PRECAUTIONS
The product contains sodium azid (<0,1%) to prevent bacterial growth. Do not empy into drains. Avoid contact with skin and eyes.

For more information refer to Material Safety Data Sheet

RECONSTITUTION
Always allow reagent, Diluent and CaCl2 to reach room temperature before reconstitution.

GHI131 Ownen’s PT reagent can be reconstituted in two ways; referred to as X1 method and X2 method
- 1X method: reconstitution with Diluent. CaCl2 is then added to the the reconstituted reagent
- 2X method: reconstitution with Diluent, CaCl2 is added separately at analysis

The 1X method is more convenient and can often gain a higher throughput on many automatic instruments, but requires higher demands on system cleanliness since a CaCl2 activated reagent will be more sensitive to contamination. Therefore in some cases the 2X method is to prefer, since the reagent is not activated without CaCl2.

Reconstitution 1X-method:
For one vial GHI131 add Diluent GHI154 or CLSI CLRW type water or equivalent (ref 5) according to table below:

<table>
<thead>
<tr>
<th>GHI131-4</th>
<th>GHI131-10</th>
<th>GHI131-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td>2 mL</td>
<td>GHI154</td>
</tr>
<tr>
<td></td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mL</td>
<td></td>
</tr>
</tbody>
</table>

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 25mM CaCl2 (GHI155) according to table below

<table>
<thead>
<tr>
<th>GHI131-4</th>
<th>GHI131-10</th>
<th>GHI131-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>25mM CaCl2</td>
<td>2 mL</td>
<td>GHI155</td>
</tr>
<tr>
<td></td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mL</td>
<td></td>
</tr>
</tbody>
</table>

Reagent intended to be used the same day as reconstitution.

PT-reagent reconstituted according to 1X-method let stand for two hours after reconstitution before use.

Note this is for using the reagent the same day as reconstitution.

Changes: Note: The new IFU revision does not include any change of procedures. New article no added GHI131-10SI, GHI131-4, GHI131-20, and PT Buffer MRX150, MRX152. Information that CLSI CLRW type water can be used for reconstitution.

Note this is for using the reagent the same day as reconstitution.

For 7 days stability see Storage conditions and stability.

At rare occasions there can be small precipitations in the X1 reconstituted reagent. Primarily if the reconstituted reagent is stored refrigerated. The precipitate does not affect the analysis results and the reagent can be used as normal. But it is recommended to remove the precipitate.

Reconstitution 2X-method:
For one vial GHI131 add Diluent GHI154 or CLSI CLRW type water or equivalent (ref 5) according to table below:

<table>
<thead>
<tr>
<th>GHI131-4</th>
<th>GHI131-10</th>
<th>GHI131-20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diluent</td>
<td>2 mL</td>
<td>GHI154</td>
</tr>
<tr>
<td></td>
<td>5 mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mL</td>
<td></td>
</tr>
</tbody>
</table>

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.

Reagent intended to be used the same day as reconstitution.

PT-reagent reconstituted according to 2X-method let stand for one hour after reconstitution before use.

Note this is for using the reagent the same day as reconstitution.

For 7 days stability see Storage conditions and stability.

For both 1X- and 2X-method it is important to mix the reagent before use but it is not necessary with continuous stirring when reagent is kept on the instrument.
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.

Note: If the reconstituted reagent is supposed to be used over a period of several days it is strongly recommended to reconstitute the reagent the day prior to use to achieve 7 days stability.
The reason is that the sensitivity (SI) slightly changes the first 12-24 hours after reconstitution (See stability data in the certificate).

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-tartricate 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 2500xg 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.

Pre dilutions for plasma, citrate- and capillary blood

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

Procedure plasma
Analysis with 1X-method reagent
- 100 µl plasma diluted 1:7, pre-heated to 37°C
- 200µl PT reagent 1X, pre-heated to 37°C

Analysis with 2X-method reagent
- 100 µl plasma diluted 1:7, pre-heated to 37°C
- 100µl PT reagent 2X, pre-heated to 37°C
- Mix plasma and PT reagent
- Add 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. The coagulation time that is the interval from the last addition until coagulation occurs. The PT-INR is calculated using equation INR = (PT/MNPT)0,4.

Analysis results:
Normal range INR 0,92-1,20 (70-130%)
Optimal oral anticoagulation treatment with thrombosis profilax:
INR 2-3 (15-25%),(ref 3)

CALIBRATION
Calibrate the measuring system according to local routines or guidelines for Owrens PT.

QUALITY CONTROL (Not included in the kit)
For reliable quality control of the performance it is recommended to use MediRox control plasma (2 level controls GHI 162-GHI170 or 3 level controls MRX170- MRX183) and at a frequency in accordance with good laboratory practise.

LIMITATIONS AND INTERFERING SUBSTANCES
The PT results may be affected by insufficient blood sampling with shifted ratio of sodium citrate to patient plasma or by interfering substances such as heparin, EDTA and vitamin K.

PT is not affected by substances in concentrations up to:

- Heparin UFH* 0,5 IU/mL.
- Triglycerides 10g/L.
- Bilirubin 0,3 g/L.
- Hemoglobin 10g/L.

* Means unfractionated Heparin and use of dilution buffer MRX150/GHI150. When using buffer with Polybrene MRX152/GHI152 the reagent is not affected by unfractionated Heparin up to 1 IU/mL.

It is recommended that each laboratory should establish its own heparin therapeutic range.
The GHI131 reagent is enriched with bovine FV and fibrinogen making it insensitive to variations of the same in patient samples.

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>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1,00</td>
<td>0,7</td>
<td>0,5</td>
<td>0,9</td>
</tr>
<tr>
<td>Level 2,30</td>
<td>1,3</td>
<td>1,2</td>
<td>1,9</td>
</tr>
</tbody>
</table>

REFERENCES
2. Ref.2 Boswell A M H P vs den. 1991. The significance of the international Normalised Ratio (INR) for oral anticoagulant therapy. JIFCC 5; 146-53