MediRox

CE

Revision: 2019-01-14
Changes: Storage conditions and stability: Change in stability for reconstituted reagent stored at 15-25°C from 7 days to 5 days;
Clarified handling of precipitation;
Clarified information regarding reconstitution and stability
Supersedes: 2018-08-20

MRX Owren´s PT
Art. No: GHI131-4, GHI131-10, GHI131-10SI, GHI131-20

INTENDED USE
MRX Owren´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 Owren´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 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 concentration (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 insensitiveness for Heparin can be enhanced by using sample dilution buffer with polybrene (MRX152 or GHI152).

PT activity was previously expressed in percentage of the normal plasma activity[1] but is more often reported as INR due to inconsistency in PT % results between laboratories. PT Owren´s is typically not reported as seconds.

Owren´s PT activity is expressed in INR (International Normalized Ratio) which is the quotient between the coagulation time for a sample and the coagulation time for a normal plasma (MNPT) raised to the method's ISI-value (International Sensitivity Index)[2]

- $INR = (PT/MNPT)^{ISI}$
- ISI = Lot specific International Sensitivity Index for reagent/instrument system.The ISI value for the PT reagent is obtained by calibrating with reference calibrators.

ISI and MNPT should be established for each lot of reagent on every individual measuring system according to local practise.

PRODUCT DESCRIPTION
MRX Owren´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 of the coagulation factors II, VII and X.

Package:

GHI131-4	10 x 4 mL (vial size 22 x 40 mm)
GHI131-10	10 x 10 mL (vial size 22 x 49.5 mm)
GHI131-10SI	10 x 10 mL (vial size 30 x 50 mm)
GHI131-20	10 x 20 mL (vial size 27.5 x 60 mm)

Material needed but not included in the kit:

- GHI155, GHI155-2, GHI155-5, GHI155-10 25 mM $CaCl_2$
- MRX150 PT buffer or GHI150 Owren´s buffer or MRX152 PT buffer with polybrene or GHI152 Owren´s buffer with polybrene
- GHI154, GHI154-2, GHI154-10 diluent or CLSI CLRW type water or equivalent[3]

PRECAUTIONS
The product contains sodium azid (<0,1%) to prevent bacterial growth. Do not empty into drains. Avoid contact with skin and eyes.
For more information refer to the Material Safety Data Sheet

RECONSTITUTION
It is important to let the reagent, diluent GHI154 (or CLSI CLRW type water or equivalent) and $CaCl_2$, reach room temperature (15-25°C) before reconstitution. There is a minor risk of precipitation in reagents reconstituted with solutions colder than 15°C, for the 1X method. See information about precipitation under Reconstitution 1X method.

MRX Owren`s PT reagent can be reconstituted using two different methods; the 1X method and 2X method.

1X method:

Reconstitution with diluent GHI154 (or CLSI CLRW type water or equivalent)

$CaCl_2$ is added to the reconstituted reagent

2X method:

Reconstitution with diluent GHI154 (or CLSI CLRW type water or equivalent.)

$CaCl_2$ is added separately during analysis in the instrument

The 1X method is more convenient and can often gain a higher throughput on many automatic instruments, but requires higher demands on system cleanness as an $CaCl_2$ activated reagent will be more sensitive to contamination. Thus, usage of a PT reagent, inactivated due to the absence of $CaCl_2$ (2X method), is preferable in some cases.

Reconstitution 1X-method:
Add room-tempered (15-25°C) diluent GHI154 or CLSI CLRW type water or equivalent[5] to the reagent vial, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
Diluent GHI154 or CLSI CLRW type water or equivalent	2 mL	5 mL	10 mL

Incubate for 10 minutes at 15-25°C; mix at the beginning, in the middle and at the end of this period. The reagent will dissolve into a slightly opaque colourless liquid.

Add room-tempered 25mM $CaCl_2$ (GHI155) to the vial with reconstituted reagent, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
25mM $CaCl_2$ GHI155	2 mL	5 mL	10 mL

If the reagent reconstituted according to 1X method is intended to be used the same day as it is reconstituted, wait for two hours after reconstitution, before use of the reagent.

If the reagent is intended to be used over several days, it is strongly recommended to reconstitute the reagent the day before use, see section; Storage conditions and stability.

It is important to mix the reagent before use, but it is not necessary with continuous stirring when the reagent is kept in the instrument.

On rare occasions small precipitations can occur in the 1X reconstituted reagent, primarily if the reconstituted reagent is stored refrigerated or if the reagent, diluent and $CaCl_2$ are colder than 15°C at the time reconstitution. The precipitate does not affect the result or performance of the reagent, however it is recommended to remove the precipitate, with a clean pipette tip, before use.

Reconstitution 2X-method:
Add room-tempered (15-25°C) diluent or CLSI CLRW type water or equivalent[3] to the reagent vial, according to the table below.

	GHI131-4	GHI131-10 GHI131-10SI	GHI131-20
Diluent GHI154 or CLSI CLRW type water or equivalent	2 mL	5 mL	10 mL

Incubate for 10 minutes at 15-25°C; mix at the beginning, in the middle and at the end of this period. The reagent will dissolve into a slightly opaque colourless liquid.

If the reagent reconstituted according to 2X method is intended to be used the same day as it is reconstituted, wait for one hour after reconstitution, before use of the reagent.

If the reagent is intended to be used over several days, it is strongly recommended to reconstitute the reagent the day before use, see section; Storage conditions and stability.

It is important to mix the reagent before use but it is not necessary with continuous stirring when reagent is kept in the instrument.

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STORAGE CONDITIONS AND STABILITY

Unopened reagent stored at 2-8°C is stable until the expiration date shown on the vial.

Stability after reconstitution
7 days at 2-8°C, in the closed original vial
5 days at 15-25°C, in the closed original vial

Storage at room temperature (15-25°C) is recommended for reagents reconstituted according to 1X-method.

Note: If the reconstituted reagent is intended to be used over several days it is strongly recommended to reconstitute the reagent the day before use to achieve 5 days stability (storage at 15-25°C) or 7 days stability (storage at 2-8°C). This recommendation is based on the change of International Sensitivity Index (ISI) during the first 12-24 hours after reconstitution, followed by a stabilization of the ISI value for 5 days (storage at 15-25°C) and 7 days (storage at 2-8°C) after reconstitution.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection and storage be carried out in accordance with CLSI instructions H21-A5[4], 9 parts of freshly drawn venous blood are collected into 1 part 0.13 M tri-natriumcitrate and 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.

Predilutions 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
- 200 µl PT reagent 1X, pre-heated to 37°C

Analysis with 2X-method reagent

- 100 µl plasma diluted 1:7, pre-heated to 37°C
- 100 µl PT reagent 2X, pre-heated to 37°C
- Mix plasma and PT reagent
- Add 100 µl 25 mM $CaCl_2$ pre-heated to 37°C

For both methods the following is applicable: mix immediately and let react at 37°C.
The coagulation time is the interval from the last addition until coagulation occurs.
The PT-INR is calculated using the following equation $INR = (PT/MNPT)^{ISI}$.

Analysis result:
Normal range INR 0.92-1.20 (70-130%)
Optimal oral anticoagulation treatment with vitamin-K antagonists:
INR 2-3 (15-25%)[5]

CALIBRATION

Calibrate the measuring system according to local routines or guidelines for Owren´s 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) at a frequency in accordance with good laboratory practise.

LIMITATIONS AND INTERFERING SUBSTANCES

The PT results may be affected by insufficient blood sampling with a 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	10 g/L
Bilirubin	0.5 g/L
Hemoglobin	10 g/L

* 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)

	CV% within series	CV% between series	CV% Total
Level 1,00	0.7	0.5	0.9
Level 2,30	1.5	1.2	1.9

REFERENCES

1. Besselar A M H P va den. 1991. The significance of the international Normalized Ratio (INR) for oral anticoagulant therapy. JIFCC 3; 146-53
2. Besselar A M H P va den. Lewis SM, Mannucci P M Poller L. 1993. Status of present and candidate International Reference Preparations (IRP) of thromboplastin for prothrombin time. Thromb Haemostas 69;85.
3. Clinical and Laboratory Standards Institute. Preparation and Testing of Reagent Water in the Clinical Laboratory, Fourth Edition, CLSI Document C3-A4; Vol. 26 No. 22.
4. CLSI Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays 5th Ed. CLSI document H21-A5 Vol. 28 No.5
5. Schulman S et al 1995. A comparison of six weeks with six months of oral anticoagulant therapy after a first episode of venous thromboembolism. New Eng J Med 332:1661.