CE

# Instructions for Use
# Kollagenreagens Horm®

## 1. Name of the medical device
Kollagenreagens Horm®

## 2. Intended purpose
In-vitro diagnostic medical device for determination of thrombocyte aggregation capacity by suitably trained medical personnel.

## 3. Precautions
When used as directed, no special precautions are required, beyond the general precautions in the handling of human blood as a potentially infectious specimen material.

## 4. Composition
Kollagenreagens Horm® suspension contains 1 mg/ml of native collagen fibrils from equine tendon in isotonic glucose solution, with parabenes as a preservative. The SKF solution is an isotonic glucose solution (pH 2.7-2.9).

## 5. Storage
Kollagenreagens Horm® is to be stored at 2-8°C. Its shelf life is two years.
After opening for the first time, the contents should be sealed in an airtight manner. Microbial contamination of the opened vial is to be avoided by aseptic working practices. Freezing can impair the stability of the suspension and for this reason should be avoided.

## 6. Presentation
1 test kit contains:
- 1 vial with 4 ml of Kollagenreagens Horm® suspension
- 4 ampoules of 10 ml with SKF-solution as diluting solution

## 7. Test material
The recommended test material is so-called platelet-rich plasma, obtained by centrifugation (15 min at 100 x *g* or 10 min at 150 x *g*) from citrated blood and adjusted to a thrombocyte concentration of 2.0-3.0 x $10^5/\mu l$ with low-platelet plasma. Thrombocyte concentrations of < 2.0 x $10^5/\mu l$ should be avoided, to ensure sufficient accuracy of measurement. In addition to measurement of thrombocyte aggregation in platelet-rich plasma, methods are also described in the literature that permit the use of whole blood as the test material (Marx and Schulte, 1972).

## 8. Method

### 8.1. Principle
The collagen fibrils in Kollagenreagens Horm® act as an inducer of platelet aggregation, which can be determined quantitatively by turbidimetry (Born and Cross, 1963). Aggregometric investigation is carried out in accordance with the instructions of the manufacturer of the turbidimeter.

### 8.2. General instructions for use
- Before use, the vial of Kollagenreagens Horm® suspension should be inverted several times or gently swirled. Shaking can cause tiny air bubbles to be trapped in the solution, which falsify the measurement results.
- Kollagenreagens Horm® suspension should be diluted exclusively with the SKF-solution supplied in the test kit, and never with physiological saline.
- Airtight storage is required for Kollagenreagens Horm® suspension in the opened vial, therefore it is recommended to fill a suitable, sealable container aseptically with the quantity needed for a week, and store this in a cool place (2-8°C).
- The pipetting of very small quantities of liquid (< 20 $\mu l$) should be avoided, so as not to impair the measurement accuracy. Direct-displacement micropipettes made of glass should be used if possible.
- Vigorous shaking of the reaction vessels should be avoided, because the collagen fibrils have a tendency to adhere to surfaces and this can falsify the measurement result.
- Kollagenreagens Horm® suspension should not be diluted excessively with SKF-solution (> 1:50), in order to ensure complete induction of platelet aggregation.

### 8.3. Use of Kollagenreagens Horm® in the diagnostic test
Owing to differences in laboratory equipment (different centrifuges and aggregometers) and laboratory-specific modifications of the method of aggregation, it is recommended to define the range of physiological aggregation (normal values) individually in each laboratory. The following can therefore only be recommended as a general guide:
- Dilute the Kollagenreagens Horm® suspension with the SKF-solution supplied in the test kit to a concentration of 1:10 and add it as inducer solution to the platelet-rich plasma (adjusted to 3.0 x $10^5/\mu l$) also in 1:10 ratio.
- Depending on the particular turbidimeter used, ensure there is a sufficient volume in the measuring vessel.
- Determine the percentage aggregation by means of turbidimetry. Low-platelet plasma of the same patient in each case should be used as reference for the turbidimetric measurement of thrombocyte aggregation.
- It may be advisable to prepare several dilution steps of Kollagenreagens Horm® suspension (e.g. 1:10, 1:20, 1:30) and compare the measurement curves of the aggregometer.

## 9. Internal Quality Control
Kollagenreagens Horm® is characterised by high, stable activity, good reproducibility of the measured results and high productivity.
The manufacturer's quality management ensures that the thrombocyte-specific effect is the same in each batch of Kollagenreagens Horm®. Platelet-rich plasma of healthy trial subjects is used as reference.
So far, no international or generally recognised standard has been established for thrombocyte aggregation induced by collagen fibrils. For this reason it is reiterated that it is advisable for each laboratory to define the normal physiological values of the aggregation, taking into account the operating instructions for the respective aggregometers.
The guidance on use given in Section 8.3. ensures that there is an excess of collagen fibrils, which gives accurate and reproducible measurement results even if the aggregation capacity of the thrombocytes is disturbed (e.g. through taking acetylsalicylic acid).

## 10. References

Marx R, Schulte F: Über einen klinischen Schnelltest zur Erfassung von hereditären und erworbenen Thrombopathien [A quick clinical test for the detection of hereditary and acquired thrombopathies]. *Blut* 3:137-141, 1972

Born GVR, Cross MJ: The aggregation of blood platelets. *J Physiol* 168: 178-195, 1963

***Additional bibliography (an extract):***

Bloom AL *et al.* (eds): *Haemostasis and Thrombosis*. 3rd ed., Churchill Livingstone, 1994

Hathaway WE, Goodnight SH (eds): *Disorders of Hemostasis and Thrombosis: A Clinical Guide*. 2nd ed., McGraw-Hill, 2000

Kinlough-Rathbone RL *et al.*: Mechanisms of platelet shape change, aggregation and release induced by collagen, thrombin or A23,187. *J Lab Clin Med* 90: 707-719, 1977

Michelson AD (ed): *Platelets*. Academic Press New York, 2002

Von Willebrand Factor potency determination by collagen binding assay, a collaborative study for the establishment of a Eur. Pharm. Method. *Pharmeuropa* 14: 315-327, 2002



**11. Manufacturer**
NYCOMED Austria GmbH
St. Peter-Strasse 25
A-4020 Linz
Tel. +43.732.6919-0

**12. Distributor**
NYCOMED Pharma GmbH
Edisonstrasse 16
D-85716 Unterschleissheim
Tel. +49.89.370037-0
KRH@nycomed.com