INTENDED USE:
The kit is proposed for the measurement of Factor II (FII or Prothrombin) activity in human citrated plasma in a clotting assay, triggered with calcium thromboplastin. This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:
The method is a clotting assay where all the clotting factors are present (constant and in excess, brought by the deficient plasma), except for FII, which is brought by the diluted tested plasma, and clotting is triggered with calcium thromboplastin. FII is the limiting factor and clotting time is inversely proportional to the concentration of FII. There is an inverse linear relationship, on a bilogarythmic graph paper, between the FII concentration and the corresponding clotting time.

ASSAY SPECIMEN:
Human plasma obtained from Trisodium Citrate anticoagulated blood.

REAGENTS:
1 vial of 1 ml (DP010A-RUO) or 6 vials of 1 ml (DP010K-RUO) of citrated human plasma, deficient for Prothrombin, immuno-depleted, lyophilized in the presence of glycine and stabilizers.

PROTOCOL:

1. If the plasma used for the calibration curve is indicated as an example only. It was;
2. Asmas, titrated for Fe diluted tested plasma,
3. ...or electromagnetic water
4. sam.
5. There is an inverse linear relationship, on a bilogarythmic graph paper, between the FII concentration and the corresponding clotting time.
6. The calibration curve must be used using the factor II
7. The calibration curve can also be established with the BIOPHEN Plasma Calibrator (#222101)

ASSAY PREPARATION AND STABILITY:
In the original package, and before any use, when stored at 2-8°C, the reagent is stable until the expiration date printed on the kit.

Reagent Preparation:

1. Restore the vials with 1 ml of distilled water; mix gently until complete dissolution of the content (vortex), let for 15 min. at room temperature (18-25°C); homogenize before each use.
2. Reagent stability following reconstitution:
   - When opened and protected from any contamination, the reconstituted plasma is stable for: 8 hours at room temperature (18-25°C)
   - 24 hours at 2-8°C
   - 2 months, frozen -8°C

Note: Plasma used for the deficient Plasma preparation were tested with registered methods and found negative for HIV antibodies. HBs Ag and HIV antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

SAMPLE COLLECTION AND PREPARATION:
Blood (9 vol.) must be collected on 0.109M trisodium citrate anticoagulant (1 vol.). Plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma must be discarded water.

Imidazole buffer (ex AR201A/AR201K/AR201L).

Normal human citrated plasma pool or Factor II calibrator (BIOPHEN Plasma Calibrator - # 222101).

Normal and Abnormal quality control plasmas, titrated for Factor II (BIOPHEN Normal Control Plasma - #223201 and BIOPHEN Abnormal Control Plasma - #223301).

Calcium Thromboplastin (such as rabbit brain thromboplastin).

QUALITY CONTROL:
The control is performed using commercially available control plasmas, titrated for FII activity. Various control plasmas are available: BIOPHEN Normal Control Plasma; (ref 223201); BIOPHEN Abnormal Control Plasma; (ref 223301). Use of quality control plasmas allows validating the calibration curve, as well as the homogeneous reactivity of the assay from run to run, and from series to series, when using a same lot of reagents.

CAUTIONS AND LIMITATIONS:

1. Sampling must be performed with great care, avoiding any blood activation. Discard any plasma presenting an unusual aspect, or any sign of activation or clotting.
2. It is recommended to perform all assays of fresh calibration points, specimen and controls successively without interruption, to obtain optimal performances of the assay.
3. For a better accuracy, samples measured ≤10% can be tested at the 1.5 dilution, and obtained results divided by 2; for samples measured >100% (or C%), the 1:20 dilution can be used and obtained results multiplied by 2.
4. For a deficient sample: check the result by testing if necessary the 1:5 dilution (the obtained concentration must then be divided by 2), and/or another sample and/or method for the test plasma; check potential associated factor(s) deficiency.
5. Thrombin inhibitors present in the tested sample may lead to an understimation of the FII concentration.

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

NORMAL VALUES:
Normal values for Factor II activity are usually > 70%.

APPLICATIONS:

1. The reagent is proposed for measuring Factor II activity, by clotting assay.
2. Lyophilized, human citrated plasma, deficient for Prothrombin, for any in vitro protocol or research study where a source of human Prothrombin deficient plasma is required.

ASSAY VARIATIONS:
The clotting times observed for this assay are obtained with Calcium Thromboplastin from Biomekrix (Calcium Thromboplastin) or from Diagnostica Stago (Neoplastin). They are expected <30 seconds for the 100% FII concentration. The obtained clotting times and assay performances can slightly vary according to the thromboplastin reagent type and lot, and the instrument used in the laboratory. Performances, as well as target values and acceptance ranges for each new lot of quality controls used, and the normal range, must then be confirmed (and adjusted if necessary) in the laboratory working conditions.

For research use only. Not for use in diagnostic procedures.