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# P3NP-ELISA

## N-Terminal Procollagen III Peptide



[www.cisbio.com/ivd-pi](http://www.cisbio.com/ivd-pi)

## Instructions for Use - ENG



Document reference: 11 – March 2024

## 1. NAME AND INTENDED USE

**P3NP-ELISA is an immuno-assay for the quantitative measurement of N-Terminal Procollagen III Peptide (PIIINP) in serum, EDTA or heparin plasma.**

The kit is intended for professional use for in vitro diagnostic.

## 2. INTRODUCTION

Procollagen type III is synthesized in fibroblasts as a biosynthetic precursor of collagen type III, and then released. The propeptides are split off in the extracellular space during the conversion into collagen. The N-terminal propeptide (PIIINP; MW 45000) is formed during this process in equimolar proportions to collagen type III and enters the circulation.

Bloodstream levels of PIIINP can therefore be used as a measurement of collagen III synthesis.

### 2.1. Clinical significance of quantitative procollagen III N-terminal peptide determination

The main collagens found in the connective tissue of the liver are types I and III. If, as a result of pathological conditions, there is active proliferation of connective tissue (fibrosis) in the liver, increasing amounts of procollagen III N-terminal peptide are formed.

The transformation of functioning liver tissue into connective tissue is detectable by means of the raised bloodstream procollagen III N-terminal peptide level. This is, for instance, the case in:

- **alcoholic or virus induced forms of liver fibrosis and cirrhosis**
- **cases of nonalcoholic steatohepatitis (NASH) [1].**

**Also, the European S3 Guidelines on the systemic treatment of *psoriasis vulgaris* recommend that PIIINP measurements be included in laboratory controls to monitor the risk of liver fibrosis in psoriasis patients receiving methotrexate [2].**

### 2.2. Pathological values

Pathological conditions affecting the liver, which are associated with active proliferation of connective tissue, give rise to raised serum PIIINP values. Therefore, the transformation of functioning liver tissue into connective tissue can be detected by measuring P3NP in the serum. According to the degree of severity of the disease, PIIINP in the serum is raised in chronic active hepatitis, fibrosis and cirrhosis of the liver.

Levels in chronic persistent hepatitis are generally in the normal range; procollagen III peptide may be raised in degeneration of the liver.

In acute hepatitis procollagen III peptide in the serum is also raised.

There are, however, other conditions in which PIIINP is raised without a detectable change in the liver (e.g. pulmonary fibrosis [3], rheumatic disorders, myocardial infarction [4], acromegaly, multiple trauma).

The diagnostic importance is in monitoring the course of the disease. There is a good correlation with histological findings in fibrosis and cirrhosis.

### 3. PRINCIPLE

The P3NP-ELISA kit is a **one-step sandwich colorimetric ELISA**-type immunoassay. A monoclonal antibody, immobilized on the microplate, captures the PIIINP proteins contained in the calibrators and samples and the bound proteins are then recognized by a second monoclonal antibody conjugated to HRP (Horseradish Peroxidase). The unbound reagents are eliminated by washing. Then, the colorimetric reaction is started by the addition of an HRP substrate, TMB (3,3',5,5' Tetramethyl-benzidine). The reaction is stopped by addition of an acid solution, the optical density (OD) of each well is read at 450 nm. The OD values are proportional to the PIIINP protein concentrations contained in the calibrators and samples.

### 4. REAGENTS

Each kit contains enough reagents for 96 tests. The expiry date is marked on the kit external label.

**Before opening, all reagents must be stored at 2-8°C until the expiry date.**

**After opening, the kit can be used for 6 weeks when reagents are stored as described below:**

REAGENTS	SYMBOLS	QUANTITY	STORAGE AFTER OPENING
<b>MICROPLATE:</b> Ready for use. Anti-PIIINP mouse monoclonal antibody immobilized onto the surface of the wells.	<b>MICROPLATE</b>	1 plate (96 wells) (foil pouch with dessicant)	After opening, any unused strips may be stored for 6 weeks at 2-8°C in the plastic bag supplied, with dessicant, properly sealed.
<b>CONJUGATE:</b> Ready for use. Buffered solution containing mouse monoclonal anti-PIIINP antibody linked to horseradish peroxidase, mouse immunoglobulins, stabilizers, and preservative.	<b>CONJ</b>	1 x 12 mL vial	After opening, the solution should be stored at 2-8°C and used within 6 weeks.
<b>DILUENT – CALIBRATOR 0 (CAL 0):</b> Ready for use. Buffered solution containing bovine proteins, preservatives and a yellow-orange dye.	<b>DIL CAL0</b>	1 x 35 mL vial	After opening, the solution should be stored at 2-8°C and used within 6 weeks.
<b>CALIBRATORS (CAL 1 – CAL 5):</b> lyophilized. Lyophilized buffered solution containing fetal calf serum, bovine proteins, preservatives and a yellow-orange dye. 2.5 – 5 – 10 – 20 – 30 µg/L* Reconstitute with 1 ml distilled water, replace the cap, invert several times and vortex to ensure complete reconstitution.	<b>CAL</b>	5 vials qs 1 mL	After reconstitution, do not store for more than 3 hours at room temperature. Store at 2-8°C for maximum 1 week or divide into aliquots and freeze at < -16°C for a period of 6 weeks (maximum of 1 freezing step).
<b>CONTROLS 1 &amp; 2 (Low and High):</b> lyophilized. Lyophilized human plasma +/- fecal calf serum Reconstitute with 0.25 ml distilled water, replace the cap, invert several times and vortex to ensure complete reconstitution. The actual acceptance limit values are printed on the vial label.	<b>CONTROL</b>	1 vial each qs 0.25 mL	After reconstitution, do not store for more than 3 hours at room temperature. Store at 2-8°C for maximum 1 week or divide into aliquots and freeze at < -16°C for a period of 6 weeks (maximum of 1 freezing steps).
<b>PBS BUFFER:</b> tablets. Phosphate buffered saline. Solubilize 1 tablet into distilled water to prepare 100 ml PBS buffer. WARNING! To prepare the wash buffer solution, add 0.3 mL of TWEEN20 reagent to each 100 mL of PBS buffer and mix slowly. (The 2 remaining tablets are provided if needed)	<b>BUF WASH</b>	4 blisters of 3 tablets each (quantity sufficient to prepare 1 liters of wash buffer solution)	After opening from the blister, tablets have to be immediately solubilized
<b>TWEEN20:</b> Tween-20 solution.	<b>TWEEN 20</b>	1 x 10 mL vial	2-8°C until the expiry date.
<b>SUBSTRATE:</b> Ready for use. Reagent containing 3, 3', 5, 5' tetramethylbenzidine (TMB).	<b>SUBS TMB</b>	1 x 15 mL vial	2-8°C until the expiry date.
<b>STOP SOLUTION:</b> Ready for use. 0.5 M sulphuric acid solution.	<b>STOP SOLN</b>	1 x 22 mL vial	2-8°C until the expiry date.
<b>ADHESIVE FILM FOR MICROPLATE</b>		2	
<b>PLASTIC BAG</b>		1	

\*The values indicated above are only target values. The true value of each calibrator is shown on its label.

## 5. PRECAUTIONS FOR USE

### 5.1. Safety measures

- The raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and have been found to be negative for anti-HIV 1, anti-HIV 2 and anti-HCV antibodies and the HBs antigen. However, as it is still impossible to strictly guarantee that such products are incapable of transmitting hepatitis, the HIV virus or any other viral infection, all raw materials of human origin, including the samples to be assayed, must be treated as potentially infectious.
- Wear disposable gloves while handling kit reagents or samples and wash hands thoroughly afterwards. Avoid splashing.
- Decontaminate and dispose of samples and all potentially contaminated materials as if they contained infectious agents. The best decontamination method is autoclaving for a minimum of one hour at 121.5°C.
- When disposing of waste, dilute thoroughly to prevent the formation of such products.



**DIL CALO CAL**

**WARNING**  
**H317: May cause an allergic skin reaction**

### 5.2. Handling precautions

- Do not use kit components beyond their expiry date.
- Do not mix reagents from different batches. Reagents batches numbers are attributed to a specific kit lot. Information is detailed in the Quality Control Report sheet.
- Avoid any microbial contamination of the reagents and water. Comply with the incubation times.

## 6. SAMPLE COLLECTION AND PREPARATION

### 6.1 Pre-analytical

- This assay is intended for the measurement of PIIINP in **human serum, EDTA or heparin plasma** samples.

Two independent studies were performed to compare the results obtained with the P3NP-ELISA kit between EDTA plasma/serum paired samples (n=39) and heparin plasma/serum paired samples (n=35)

A Passing-Bablok regression analysis was applied to those samples, yielding the following equations:

- ✓ **[Plasma EDTA Conc µg/L] = 0.997 x [Serum Conc µg/L] - 0.13 µg/L**, *Pearson correlation coefficient r = 0.99*  
*The 95% confidence intervals for the slope and the intercept were 0.92 to 1.08 and -0.68 to 0.39 µg/L respectively for the 39 patient samples having PIIINP concentrations ranging from 3.42 to 25.0 µg/L in serum*
- ✓ **[Plasma Heparin Conc µg/L] = 0.975 x [Serum Conc µg/L] + 0.11 µg/L**, *Pearson correlation coefficient r = 0.99*  
*The 95% confidence intervals for the slope and for the intercept were 0.88 to 1.06 and -0.92 to 0.72 µg/L for the 35 patient samples having PIIINP concentrations ranging from 4.44 to 29.0 µg/L in serum*
- Serum or EDTA plasma samples can stand at room temperature (18-25°C) for a maximum of 4 hours before assaying the PIIINP concentration.
- Serum or EDTA plasma samples can be used immediately or stored at 2-8°C for up to 3 days. If the test is not run within 3 days following sampling, samples must be aliquoted and stored frozen at -20°C.
- After thawing, plasma or serum must be carefully mixed. Avoid successive freezing and thawing.

### 6.2 Pre-dilution of samples and controls (1/11)

- **All the samples and the kit control must be pre-diluted 11 times** in the diluent provided in the kit (e.g. 30 µL sample + 300 µL diluent **DIL CALO**) before being assayed. Gently mix the mixture using a Vortex mixer.
- If high levels of PIIINP are suspected, additional dilutions may be necessary.

## 7. ASSAY PROCEDURE

### 7.1 Equipment required

- Precision micropipettes or similar equipment with disposable tips for distribution of 20, 50, 100, 200 and 1000 µL. Calibration of these must be regularly checked.
- Distilled water.
- Disposable plastic tubes.
- Vortex mixer.
- Microplate washer (optional).
- Microplate shaker.
- Microplate reader, capable of measuring absorbance at 450 nm. As an option, the reader may be fitted with a filter capable of reading the absorbance at a wavelength of between 610 nm and 650 nm (620 nm recommended). This second reading makes it possible to correct the microplate's imperfections.

### 7.2 Protocol

- All the reagents must be brought to room temperature (18-25°C) at least 30 minutes before their use. The reagents are taken up and distributed into wells at room temperature (18-25°C).
- Each calibrator, control or sample must be tested in duplicate.
- Determine the number of wells required for the assay and remove any unused strips. Store at 2-8°C in the plastic bag supplied for this purpose, with desiccant, and properly sealed.
- Reconstitute the vials of calibrators and control. Carefully check that all the lyophilisate is dissolved, and use within an hour following reconstitution.

#### 7.2.1 Preparation of the Wash solution **WASH**

- To obtain reliable and reproducible results, it is recommended that the washing steps be performed as indicated; the residual washing solution volume must be as low as possible. The use of a microplate washer is recommended.

**CAUTION!** The **BUF WASH** tablets are intended to prepare a phosphate buffered saline solution. It is mandatory to add 0.3 mL **TWEEN 20** solution for each 100 mL of phosphate buffered saline solution to constitute the wash buffer solution **WASH** mentioned in the protocol during the washing steps.

- Solubilize 1 **BUF WASH** tablet into distilled water to prepare 100 mL PBS buffer.
- Add 0.3 mL of **TWEEN 20** reagent to each 100 mL of solution and mix slowly.
- Label the recipient containing this wash solution as **WASH**. This solution is stable for 1 week at 2-8°C.

#### 7.2.2 Instructions - Comply with the order for addition of reagents

*See last page for laboratory protocol card. It is necessary to fully read the package insert in details before using the laboratory protocol card.*

If applicable, dilute samples with presumed high PIIINP concentrations (> 30 µg/L) using the diluent **DIL CALO** reagent supplied in the kit.

1. Reconstitute calibrators (1 mL **CAL**) and controls (0.25 mL **CONTROL**) by adding distilled water, replace the cap, reverse several times the vial and vortex to insure complete reconstitution.

**Note: calibrators are ready-to-use, DO NOT pre-dilute them**

2. Prepare and number a sufficient quantity of test tubes to perform a pre-dilution of samples and controls
3. Determine the number of microtiter well strips required for the assay. Remove unused strips from the frame holder and store them at 2-8°C in the adhesive bag, properly sealed.

#### 4. Pre-dilute samples and controls to 1:11

- a. Dispense 300 µL of diluent **DIL CALO** into the plastic tubes
- b. Add 30 µL of each sample or controls to each tube and gently mix with a vortex-type mixer

**Note: Pre-diluted samples and controls can be stored for 1 hour at room temperature (18-25°C) before the assay (>1 h not tested)**

5. Add 100 µL of calibrators **CAL**, controls **CONTROL** and samples to the appropriate wells in duplicate.

6. Dispense 100  $\mu\text{L}$  of antibody-HRP conjugate **CONJ** in wells.
7. Cover with the adhesive film and incubate for **3h** at room temperature (18-25°C) **under orbital agitation at 700 rpm**.
8. Wash the wells as follows:
  - a. Remove the content of the wells
  - b. Distribute 300  $\mu\text{L}$  of wash solution **WASH**, prepared as described in chapter 7.2.1
  - c. Repeat steps a. and b. 2 times more for a total of 3 washing cycles.
  - d. Finish by aspirating. The residual washing solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.
9. Dispense **100  $\mu\text{L}$**  of TMB substrate **SUBS TMB** in all wells.
 

**Important: Start the 15 min Incubation Time from the first dispensed well.**
10. Cover with the adhesive film and complete the **15 min** incubation at room temperature (18-25°C) **WITHOUT agitation**. Incubation in darkness is not necessary.
11. Stop the reaction by adding **100  $\mu\text{L}$**  stop solution **STOP SOLN** to all wells.
12. Remove the adhesive film and measure the absorbance (OD) within 30 minutes after adding the stop solution:
 

It is recommended to clean the outer bottom of the wells with a lint-free soft tissue to eliminate possible fingerprints or smudges

  - Perform a read at 450 nm (Optional: perform a read at a wavelength of 620 nm)

## 8. QUALITY CONTROL

Good Laboratory Practices (GLP) require that quality control samples be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

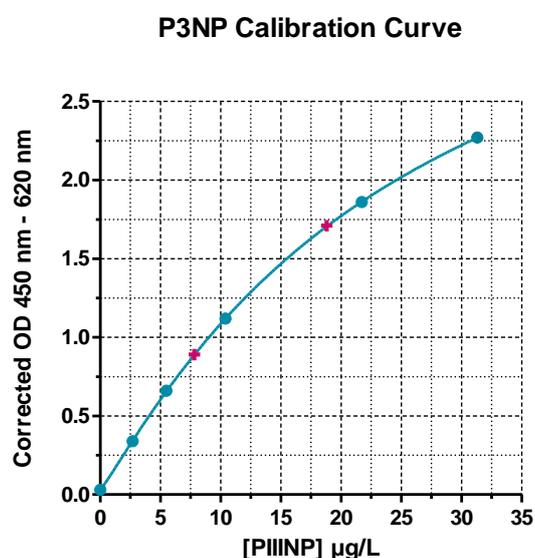
## 9. RESULTS

1. Optional OD correction\*: subtract readings at 620 nm from the readings at 450 nm.
2. For each duplicate, calculate the mean absorbance (OD) of calibrators, controls and samples.
3. Construct a calibration curve by plotting the (corrected\*) mean OD values at 450 nm of calibrators (y-axis) against their concentration (x-axis) indicated on the vial.
4. The **4-parameters Logistic (4-PL)** mathematical fitting model is recommended for the calibration curves. Other data reduction functions may give slightly different results.

Read the values of the samples from the curve, correcting them by the additional dilution factor if required. The 1:11 predilution ratio is already calculated in calibrator concentrations.

Example of assay data: for illustration only and must under no circumstances be substituted for results obtained in the laboratory.

	Concentration $\mu\text{g/L}$ (see vials)	Corrected* OD 450-620 nm
<b>CAL0</b>	0	0.02
<b>CAL1</b>	2.7 (example)	0.34
<b>CAL2</b>	5.5 (example)	0.66
<b>CAL3</b>	10.4 (example)	1.12
<b>CAL4</b>	21.7 (example)	1.86
<b>CAL5</b>	31.3 (example)	2.27
<b>CONTROL 1</b>	7.8 (example)	0.89
<b>CONTROL 2</b>	18.8 (example)	1.71



## 10. LIMITATION OF THE PROCEDURE

- Samples presenting cloudiness, haemolysis, hyperlipemia or containing fibrin may give inaccurate results.
- Do not extrapolate sample values beyond the last standard. Dilute the samples concerned and retest.

## 11. PERFORMANCE CHARACTERISTICS

### 11.1 Measurement Range of the assay

The samples must be measured in the range between the lower limit of detection and the highest concentration of the calibration range.

### 11.2 Traceability

The assigned PIIINP values of the P3NP-ELISA kit are expressed in micrograms per liter ( $\mu\text{g/L}$ ) and are standardized onto an internal standard made from serum human samples traceable to a quantitative reference method.

### 11.3 Precision

#### 11.3.1 Intra-Assay

The intra-assay (within-run) variation was determined by 31 measurements of 3 serum samples covering the whole measuring range of the calibration curve.

**Within-Run Precision**

Sample	1	2	3
n	31	31	31
Mean value ( $\mu\text{g/L}$ )	5.2	13.4	25.5
CV (%)	2.2	2.4	3.6

#### 11.3.2 Inter-Assay

The Inter-assay (between-run) variation was determined using 3 serum samples measured in 8 runs in duplicate.

**Between-Run Precision**

Sample	1	2	3
n	8	8	8
Mean value ( $\mu\text{g/L}$ )	5.3	13.1	25.0
CV (%)	6.5	8.0	7.3

### 11.4 Detection limit

- The Limit of Detection (LOD or analytical sensitivity) of the P3NP-ELISA kit is defined as being the lowest detectable concentration that differs from zero with a probability of 95% calculated by adding 2 standard deviations to the mean of 30 replicate analysis of the zero calibrator (CAL0).

**Analytical Sensitivity (Limit of Detection)**

LOD $2\sigma$	0.036 $\mu\text{g/L}$
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- The Limit of Quantitation (LOQ or functional sensitivity) of the P3NP-ELISA kit is defined as being the concentration measured by the imprecision profile at a between-runs CV equal to 12.5%. It was evaluated by testing 9 serum specimens in duplicate in 8 runs. The mean, standard deviation, and %CV were then determined for each sample and a 3-parameter power variance function was used for fitting.

**Functional Sensitivity (Limit of Quantitation)**

### 11.5 Antigen recovery

PIIINP solutions from calibrators 2 to 5 were mixed 1:1 to 2 serum samples pools with various initial PIIINP concentrations. Each sample (non-spiked and spiked) was assayed in duplicates in one run. PIIINP concentrations were measured and the recovery percentages were calculated.

#### Recovery

Sample	1	2
Concentration (µg/L)	3.0	7.1
Mean recovery (%)	91	95
Range of recovery (%)	88 – 92	92 – 97

### 11.6 Dilution – Linearity

Studies were performed to evaluate the linearity of the assay using 4 serum samples of different concentrations. The samples were assayed as neat and serially diluted with DIL-CAL0 (dilution factor down to 1:16).

#### Dilution

Sample	3	4	5	6
Concentration (µg/L)	27.7	22.1	24.1	24.5
Range of Dilution	1:2 to 1:16	1:2 to 1:16	1:2 to 1:16	1:2 to 1:16
Mean recovery (%)	101	104	99	102
Range of recovery (%)	94 - 107	100 - 109	96 - 101	100 – 104

#### Linearity

Intercept	0.18	0.30	-0.11	0.13
Slope (Y=measured)	1.00	1.00	1.00	0.99
R <sup>2</sup>	0.99	0.99	0.99	0.99

Those results show the good linearity of the dilution test over the reported measuring range of this assay.

### 11.7 Specificity

The specificity of the assay is guaranteed by the use of two complementary monoclonal antibodies. The monoclonal antibodies used in the kit are specific for the N-terminal procollagen III peptide. The PIIINP peptide can be degraded by proteolysis into col1 fragments that are not recognized by the P3NP-ELISA kit.

### 11.8 Hook effect

No hook effect was observed with this assay, tested up to 200 µg/L.

### 11.9 Interferences

An interference study was evaluated according to CLSI EP17-A2 guideline. Measurements were performed using 4 to 6 replicates with 2 levels of sample (Low and mid-range of standard curve). Non-significant interference was defined as difference from control (unspiked sample) within ±10%. No interference was observed when plasma samples were tested with any of the following substances:

- Triglycerides from hyper-lipidemic EDTA plasma (Human sample – 743.4mg/dL TG total and half diluted)
- Triglycerides from a commercial Intralipid solution (30 mg/mL)
- Human albumin (spiked up to 60 mg/ml)
- Bilirubin (0.15 mg/ml)

- Human hemoglobin (2 mg/ml)
- Methotrexate (2 mM)
- Bile acids (up to 35 µM)

NOTE: Triton X-100 has been found to slightly interfere (-14% bias maximum) with this test when samples are supplemented with 0.1% of this substance.

**CAUTION:** The immunoassay is protected against potential interferences with **heterophilic antibodies** such as HAMA and rheumatoid factors (RF) by adding a protection. Nevertheless, we cannot assure that there will never be a false positive or negative result due to the presence of heterophilic antibodies in a patient sample.

## 12. EXPECTED NORMAL VALUES

In order to determine the normal range of P3NP-ELISA, 120 samples (Plasma EDTA) from presumed healthy donors were analyzed using the P3NP-ELISA kit.

The results given in µg/L are shown in the table below:

Mean	Median	5th percentile	95th percentile	Min	Max
4.9	4.6	2.9	8.1	2.1	13.1

It is recommended that each laboratory establish its own normal values. The values given below are indicative.

## 13. METHOD COMPARISON

A study was performed to compare the results of the P3NP-ELISA kit to RIA-gnost® PIIIP (Cisbio Bioassays) using 37 serum samples.

- The equivalence of the concentration reported by the RIA-gnost® PIIIP assay with respect to µg/L units was obtained by multiplying the results from the RIA kit (U/mL) by a factor of 8 to obtain µg/L as described in the RIA-gnost PIIIP® kit instructions.

A Passing-Bablok regression analysis was applied to these samples, yielding the following equation:

$$[\text{P3NP- ELISA}] (\mu\text{g/L}) = 0.94 \times [\text{RIA-gnost PIIIP}] (\mu\text{g/L}) + 1.12 \mu\text{g/L}$$

The Pearson correlation coefficient was  $r = 0.962$

The 95% confidence interval for the slope was 0.81 to 1.04, and the 95% confidence interval for the intercept was 0.41 to 2.00 µg/L for the 37 patient samples having PIIINP concentrations ranging from 3.23 to 24.9 µg/L (as measured by the P3NP-ELISA assay)

- Without applying a conversion factor to the RIA-gnost® PIIIP assay, the following equation was obtained:

$$[\text{P3NP- ELISA}] (\mu\text{g/L}) = 7.76 \times [\text{RIA-gnost PIIIP}] (\text{U/mL}) + 0.99 \text{ U/mL}$$

The Pearson correlation coefficient was  $r = 0.962$

The 95% confidence interval for the slope was 6.89 to 8.66, and the 95% confidence interval for the intercept was -0.04 to 1.66 µg/L for the 37 patient samples having PIIINP concentrations ranging from 3.23 to 24.9 µg/L (as measured by the P3NP-ELISA assay)

## 14. BIBLIOGRAPHY

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# P3NP-ELISA



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## LABORATORY PROTOCOL CARD

Do not use this card without having read the whole package insert.

↪ If applicable, dilute samples with presumed high PIIIINP concentrations using the diluent **DIL** **CALO** reagent supplied in the kit.

### 1. RECONSTITUTE calibrators (1mL) and controls (0.25 mL) with dH<sub>2</sub>O

**CAL** **CONTROL**

**Note: calibrators are ready to use, DO NOT pre-dilute them**

### 2. PREDILUTE to 1:11 samples and controls

**DIL** **CALO** **SAMPLE** **CONTROL**

1:11 pre-dilution

Prepare a sufficient quantity of tubes to perform all the pre-dilution

↓ Dispense **300 µL** of diluent to each tube

↓ Add **30 µL** of each sample or controls into the plastic tubes and gently mix with a vortex-type mixer

### 3. ADD SAMPLES TO MICROPLATE

**SAMPLE** **CAL** **CONTROL**

+ 100 µL

↓ Add **100 µL** of calibrators, pre-diluted controls and samples to the appropriate wells in duplicate.



### 4. DISPENSE CONJUGATE

**CONJ**

+ 100 µL

↓ Dispense **100 µL** of antibody-HRP conjugate in wells.



### 5. INCUBATE

Cover with the adhesive film and incubate for **3h** at room temperature (18-25°C) **under agitation at 700 rpm**

3 h  
↕ 700 rpm



### 6. WASH (see 7.2.1)

**WASH**

3 x 300 µL

Prepare wash solution = 1 tablet + 100 mL dH<sub>2</sub>O + 0.3 mL Tween20 per 100 mL

Wash the wells for **3 cycles** **Aspirate → Distribute 300 µL of wash solution**

Finish by aspirating. The residual solution volume must be as low as possible. It is possible to gently tap the plate upside down to remove the residual liquid.



### 7. DISPENSE SUBSTRATE

**SUBS** **TMB**

+ 100 µL

↓ Dispense **100 µL** of TMB substrate in all wells **and start the 15 min incubation from the first dispensed well.**



### 8. INCUBATE

Cover with the adhesive film and complete the **15 min** incubation at room temperature (18-25°C) **without agitation**. Incubation in darkness is not necessary.

15 min



### 9. DISPENSE STOP SOLUTION

**STOP** **SOLN**

+ 100 µL

↓ Dispense **100 µL** of STOP solution in all wells



### 10. READ

450 nm

↪ Perform a read at **450 nm** within 30 min – Use a **4PL fit** for data interpolation

Optional: perform a read at a wavelength of 620 nm





**FRA**

**Modifications par rapport à la version précédente :**

Nouveau logo Revvity; mise à jour de l'adresse email de l'assistance "in vitro"

**ENG**

**Changes from the previous version:**

New Revvity logo; update of the IVA email address.

**DEU**

**Änderungen gegenüber der Vorgängerversion:**

Neues Revvity-Logo; aktualisierte E-Mail-Adresse für "In-vitro"-Support

**SWE**

**Ändringar från föregående utgåva:**

Ny Revvity-logotyp; uppdaterad e-postadress för in vitro-support

FRA

ENG

DEU

SWE

	Explication des symboles	Explanation of symbols	Erläuterung der Symbole	Symbol förklaring
	Limite de température	Temperature limitation	Temperaturbegrenzung	T°-gräns vid förvaring
<b>LOT</b>	Code du lot	Batch code	Chargencode	Lotnr.
	Utiliser jusqu'au	Use by	Verwendbar bis	Används senast
	Consulter la notice d'utilisation	Consult instructions for use	Das Handbuch zu Rate ziehen	Läs bruksanvisningen
<b>IVD</b>	Dispositif médical de diagnostic in vitro	In vitro medical device	In-VitroDiagnostische Anwendung	Medicinteknisk produkt avsedd för in vitro-diagnostik
	Fabricant	Manufacturer	Hersteller	Tillverkad av
<b>REF</b>	Référence du catalogue	Catalogue number	Katalog Nr.	Referens
	Suffisant pour	Sufficient for	Ausreichend für	Antal rör
	Conserver à l'abri de la lumière du soleil	Keep away from sunlight	Vor Sonnenlicht schützen	Utsätt inte för direkt sol i jus
	Risques biologiques	Biological Risks	Biogefährdung	Biologisk risk
<b>CONJ</b>	Conjugué	Conjugate	Komplex	Konjugat
<b>CAL</b>	Calibrateur	Calibrator	Kalibrator	Kalibrator
<b>CONTROL</b>	Contrôle	Control	Kontrolle	Kontroll
<b>TWEEN 20</b>	Solution concentrée	Concentrated solution	Konzentrierte Lösung	Koncentrerad lösning
<b>MICROPLATE</b>	Microplaque	Microplate	Mikrotiterplatte	Mikroplattan
<b>BUF WASH</b>	Tampon	Buffer	Puffer	Buffert
<b>DIL CAL</b>	Diluant	Diluent	Verdünnungs-mittel	Spädningsmedel
<b>SUBS TMB</b>	Substrat	Substrate	Substrat	Substrat
<b>STOP SOLN</b>	Solution d'arrêt	Stop solution	Stopplösung	Stopplösning