Rat/Mouse PINP EIA

Enzymeimmunoassay (EIA) for the quantitative determination of N-terminal propeptide of type I procollagen (PINP) in rat/mouse serum or plasma

For Research Use Only. Not for use in diagnostic procedures.

REF AC-33F1 Σ 96

Intended Use

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The IDS Rat/Mouse PINP EIA kit is a competitive enzymeimmunoassay (EIA) for the quantitative determination of N-terminal propeptide of type I procollagen (PINP) in rat or mouse serum or plasma samples.

Summary and Explanation

An important step in the bone formation process is synthesis of type I collagen, which is the major organic component in bone matrix. During collagen synthesis, propeptides are released from both the amino- and carboxyterminal parts of the procollagen molecule (1). These propeptides are secreted into the blood circulation, and commercially available immunoassays for their measurement from human serum have been developed. Assay for human aminoterminal propeptide of type I procollagen (PINP) is probably the most specific and sensitive marker of bone formation (2). PINP is a particularly useful marker for monitoring the efficacy of osteoporosis therapy with anabolic agents (3,4), but it is also one of the best bone turnover markers for monitoring the efficacy of antiresorptive therapy (4,5).

The rat/mouse PINP assay is a specific method to determine PINP released during rat and mouse bone collagen synthesis, and it has no cross-reactivity for human PINP. The rat/mouse PINP assay can be used for determining the bone formation rate from rat and mouse serum samples, and from the culture medium in rat and mouse osteoblast cultures. Previous studies have demonstrated that PINP secretion is increased after stimulation of mouse osteoblasts by BMP-4 and estrogen in vitro (6). PINP values are also elevated after treatment with PTH in both young ovariectomized rats and old intact rats (7).

Method Description

The IDS Rat/Mouse PINP EIA kit is a competitive enzymeimmunoassay utilising a polyclonal rabbit anti-PINP antibody coated onto the inner surface of polystyrene microtitre wells. Calibrators, controls and samples are added to the wells of the microtitre plate followed by PINP labelled with biotin and the plate incubated for 1 hour at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labelled avidin is added and binds selectively to complexed biotin and, following a further wash step, colour is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microplate plate reader, colour intensity developed being inversely proportional to the concentration of PINP.

Warnings and Precautions

The IDS Rat/Mouse PINP EIA kit is *for research use only* and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IDS Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

0.5M hydrochloric acid

Stop Solution HCL contains 0.5M hydrochloric acid.
R36/38 Irritating to eyes and skin.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37 Wear suitable protective clothing and gloves.

Tetramethylbenzidine

TMB Substrate TMB contains 3,3',5,5'-tetramethylbenzidine.
R21/22 Harmful by contact with skin and if swallowed.
S36/37 Wear suitable protective clothing and gloves.

Sodium Azide

Xn. Harmful: Calibrators [CAL] and Controls [CTRL] contain sodium azide (NaN_3) >0.1% (w/w) (<1%).

R 22 Harmful if swallowed.

R52/53 Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment.

S46 If swallowed, seek medical advice immediately and show this container or label.

S36/37 Wear suitable protective clothing and gloves.

S60 This material and/or its container must be disposed of as hazardous waste.

Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Preparation of Reagents

Calibrators [CAL] and Controls [CTRL] : Calibrators [CAL] and Controls [CTRL] are supplied lyophilised. Reconstitute with 0.5 mL of distilled or deionised water, replace stopper and stand for 5-10 minutes at room temperature. Invert several times to ensure complete reconstitution.

If Calibrators or Controls are to be used more than once they must be frozen (-20°C) as soon as possible after reconstitution. When reusing Calibrators and Controls, thaw at room temperature, mix and use within 15 minutes of completion of thaw.

PINP Biotin [PINP BIOTIN] : PINP Biotin [PINP BIOTIN] is supplied lyophilised. Reconstitute with 8 mL of Sample Diluent [SAMPDIL], replace stopper and stand for 5-10 minutes at room temperature. Invert several times to ensure complete reconstitution.

Wash Solution: Add the contents of each bottle of Wash Concentrate [WASHBUF] [20x] to 950 mL of distilled or de-ionised water and mix. Store at room temperature.

All other reagents are supplied ready for use.

Allow all reagents to come to room temperature before use. Reagents should be mixed by repeated inversion before use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Reconstituted Calibrators [CAL] and Controls [CTRL] can be stored at -20°C for up to 8 weeks.

Reconstituted PINP Biotin [PINP BIOTIN] can be stored at 2-8°C for up to 8 weeks.

Unused Antibody Coated Plate strips must be returned to the foil pouch with the desiccant sachet and the pouch resealed. Store at 2-8°C for up to 8 weeks.

Wash Solution can be stored at room temperature for up to 8 weeks.

Indications of possible deterioration of kit reagents

The presence of abnormal particulate matter in any of the reagents.

A decrease in the maximum absorbance.

A shift in the slope of the curve from its normal position

Specimen Collection and Storage

The assay should be performed using serum or plasma (EDTA or heparin) specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C. Avoid repeated freeze/thaw of samples.

NB. The same specimen type should be used throughout a study.

Procedure

Materials Provided

1. **CAL 0-5 - Calibrators (REF AC-3301A - AC-3301F):**

   Lyophilised phosphate buffered saline containing rat/mouse PINP, mouse serum, goat serum, BSA and <1% sodium azide (0.025% once reconstituted)**.** The exact value of each calibrator is printed on the bottle label. 0.5 mL per bottle.

2. **MICROPLAT - Antibody Coated Plate (REF AC-3302W):**

   Microplate with polyclonal rabbit anti-PINP antibody linked to the inner surface of the polystyrene wells, 12 x 8-well strips in a foil pouch with desiccant.

3. **PINP BIOTIN - PINP Biotin (REF AC-3303):**

   Lyophilised phosphate buffered saline containing PINP labelled with biotin, and BSA. 1 mL per bottle.

4. **ENZYMCONJ - Enzyme Conjugate (REF AC-3304):**

   Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilisers and preservative. 18 mL per bottle.

5. **CTRL 1 - Control 1 (REF AC-3305A)**:

   Lyophilised rat serum diluted in PBS with BSA and <1% sodium azide (0.025% once reconstituted). 0.5 mL per bottle.

6. **CTRL 2 - Control 2 (REF AC-3305B)**:

   Lyophilised mouse serum diluted in PBS with BSA and <1% sodium azide (0.025% once reconstituted). 0.5 mL per bottle.

7. **TMB - TMB Substrate (REF AC-TMB**):

   A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 24 mL per bottle.

8. **HCL - Stop Solution (REF AC-STOP)**:

   0.5M hydrochloric acid, 14 mL per bottle.

9. **SAMPDIL - Sample Diluent (REF AC-3309)**:

   Phosphate buffered saline containing BSA and 0.05% sodium azide. 20 mL per bottle.

10. **WASHBUF 20x - Wash Concentrate (REF AC-WASHL)**:

    Phosphate buffered saline containing Tween, 50 mL per bottle.

11. **Adhesive Plate Sealers**

    8 per kit.

Materials Required but not Provided

1. Precision pipetting devices to deliver 5 µL, 45 µL and 50 µL.
2. Precision multi-channel pipettes to deliver 45 µL, 50 µL and 150 µL.
3. Microplate shaker.
4. Automatic microplate washer (optional).
5. Photometric microplate reader and data analysis equipment.

Assay Procedure

1. Add **50 µL** of each Calibrator [CAL], or Control [CTRL] to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.
2. Add **5 µL** of sample and **45 µL** of Sample Diluent [SAMPDIL] to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.

   Note: these should be dispensed within a period of 15 minutes to minimise drift.
3. Add **50 µL** of PINP Biotin [PINP BIOTIN] to all wells using a multichannel pipette.
4. Cover the plate with an adhesive plate sealer and incubate the plate on a microplate shaker (500-700 rpm) at 18-30°C for 1 hour.
5. Wash all wells three times with Wash Solution.

   a) Automatic plate wash: Set plate washer to dispense at least 300 µL of Wash Solution per well. Fill and aspirate for 3 cycles.

   b) Manual wash: Decant the contents of the wells by inverting sharply. Dispense 250 µL of Wash Solution to all wells. Decant and repeat twice.

   Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution before proceeding to the next step..
6. Add **150 µL** of Enzyme Conjugate [ENZYMCONJ] to all wells using a multichannel pipette.
7. Cover the plate with an adhesive plate sealer and incubate at 18-30°C for 30 minutes.
8. Repeat wash step 5.
9. Add **150 µL** of TMB Substrate [TMB] to all wells using a multichannel pipette.

   Note: TMB Substrate is easily contaminated. Measure out the amount of TMB Substrate required for the assay. Do not return unused TMB Substrate to the bottle.
10. Cover the plate with an adhesive plate sealer and incubate at 18-30°C for 30 minutes.
11. Add **50 µL** of Stop Solution [HCL] to all wells using a multichannel pipette.
12. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. In order to properly evaluate the performance of the assay IDS recommends that all laboratories include in each assay appropriately aliquoted and stored in-house pools in addition to the controls provided with the kit. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results

Calculate the mean absorbance for each Calibrator, Control and unknown sample. Prepare a calibration curve on semi-log graph paper by plotting the mean absorbance for each Calibrator on the ordinate against concentration of PINP on the abscissa. Read values for each control and unknown sample from the calibration curve in ng/mL.

To obtain the concentration of PINP in each sample, multiply the value read from the curve by the dilution factor used (x10).

Alternative data reduction techniques may be employed, such as automated data reduction programs, but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL curve fits are recommended.

IDS calculates results using MultiCalc (PerkinElmer) data reduction software with a 4PL fit plotting absorbance versus log concentration.

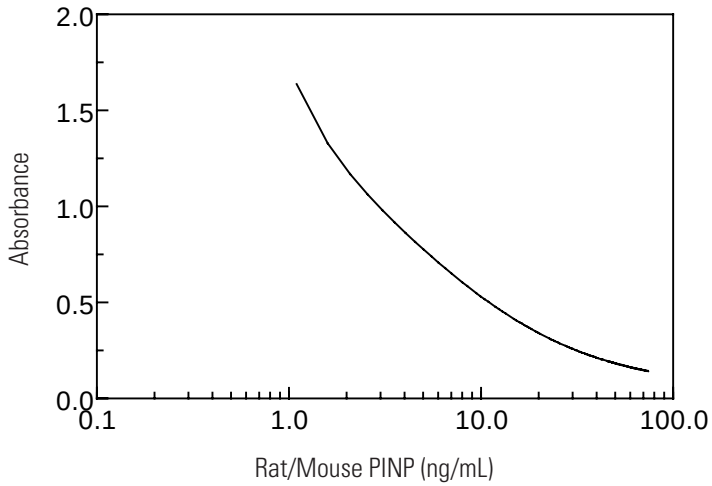
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result

Well	Description	Abs.	Mean. Abs.	%B/B_0	Result ng/mL	Corrected (x10) Result ng/mL
A1, A2	Calibrator 0 0 ng/mL	2.186 2.236	2.211	100		
B1, B2	Calibrator 1 1.1 ng/mL	1.647 1.629	1.638	74.1		
C1, C2	Calibrator 2 2.7 ng/mL	1.031 1.048	1.040	47.0		
D1, D2	Calibrator 3 9.2 ng/mL	0.569 0.546	0.558	25.2		
E1, E2	Calibrator 4 30.3 ng/mL	0.263 0.255	0.259	11.7		
F1, F2	Calibrator 5 74.4 ng/mL	0.144 0.140	0.142	6.4		
G1, G2	Sample 1	0.884 0.874	0.879	39.8	3.8	38
H1, H2	Sample 2	0.178 0.163	0.171	7.7	57.7	577

Typical Calibration Curve

This sample calibration curve is for illustration only.



Limitations of Use

The following substances have been tested and found not to interfere in the IDS Rat/Mouse PINP EIA:

Haemoglobin tested up to 500 mg/dL

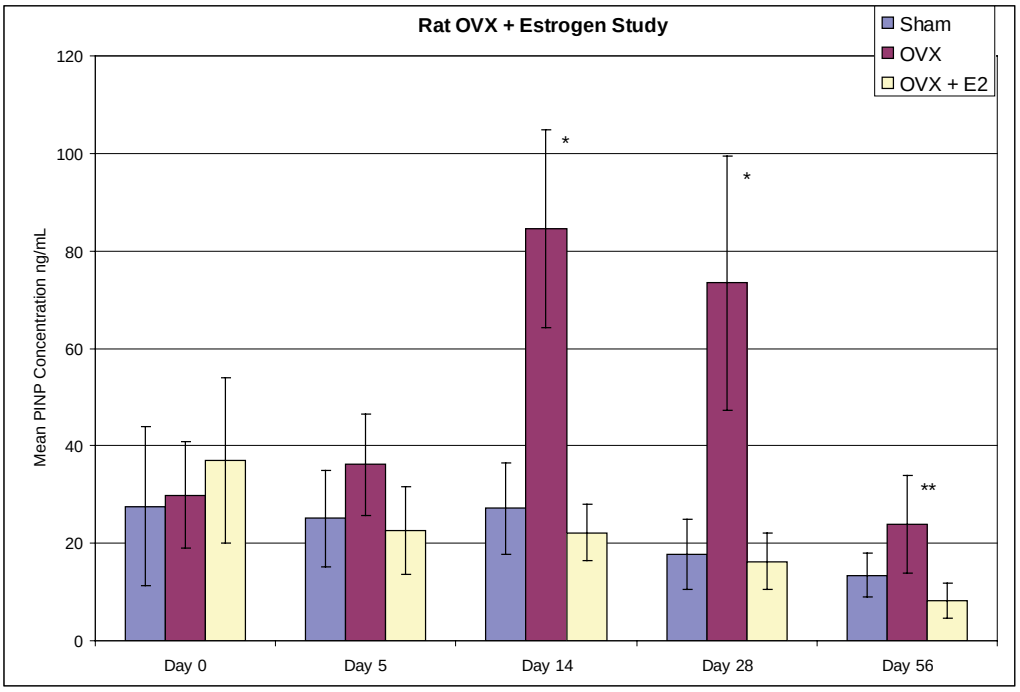
Experiment Example

Three-month-old female rats (Sprague-Dawley) were randomly allocated into three groups: (1) Sham operation (n=12), (2) ovariectomy (OVX) (n=12), and (3) ovariectomy and subsequent subcutaneous injections of 17β-estradiol (10μg/kg/day) (OVX+E2) (n=12).

PINP was determined in the Rat/Mouse PINP EIA from serum samples collected after overnight fasting before the operation and at 5, 14, 28 and 56 days after the operation.

The Rat/Mouse PINP EIA rapidly detects the increase in bone formation induced by ovariectomy. Within two weeks after surgery PINP increases to 283% of pre-operation levels. This increase in bone formation could be completely inhibited with estradiol.

Conclusion: Serum measurement of PINP in the Rat/Mouse PINP EIA detects the change in bone formation that is induced by ovariectomy of the rat.



Performance Data

Sensitivity

The sensitivity, defined as the concentration corresponding to the mean minus 2 standard deviations of 20 replicates of the zero calibrator, is 0.7 ng/mL (7ng/mL serum equivalent).

Precision

Intra assay mean (ng/mL)	n=20 % CV	Inter assay mean (ng/mL)	n=68 % CV
40	6.4	37	9.2
177	7.4	178	8.0
550	5.0	577	8.2

Recovery

Recovery was assessed by adding PINP to samples prior to assay.

Sample Concentration ng/ml	Mouse PINP added ng/ml	Measured ng/ml	Recovery ng/ml	Recovery %
69.5	100	153.7	84.2	84.2
69.5	400	506.4	436.9	109.2
			Mean	**96.7**

Linearity

Linearity was assessed by diluting a high sample with a low sample.

Sample	Measured (M) ng/ml	Expected (E) ng/ml	% M/E
Low (L)	50		
0.875L + 0.125H	93	106	88.1
0.750L + 0.250H	144	163	88.5
0.625L + 0.375H	210	219	96.0
0.500L + 0.500H	280	276	101.7
0.375L + 0.625H	298	332	89.9
0.250L + 0.750H	358	389	92.1
0.125L + 0.875H	446	445	100.1
High (H)	502		
		Mean	**93.8**

Specificity

The specificity of the antiserum was assessed with the following analytes.

Analyte	Cross-reactivity
Mouse PINP	100%
Rat PINP	100%
Human PINP (200 ng/mL)	Not detectable
Human PIIINP (20 ng/mL)	Not detectable
Rat PIIINP (10,000 ng/mL)	Not detectable

References

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Document Number: AC-33PL
Issue: 1
Date: 12 November 2007

EXP	GB	*Use By*	LOT	GB	*Batch code*
	DE	*Verwendbar bis*		DE	*Chargenbezeichnung*
	ES	*Fecha de caducidad*		ES	*Código de lote*
	IT	*Utilizzare entro*		IT	*Codice del lotto*
	FR	*Utiliser jusque*		FR	*Code du lot*
	NL	*Houdbaar tot*		NL	*Lot nummer*
	DK	*Holdbar til*		DK	*Lotnummer*
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	SK	*Použiteľné do*		SK	*Číslo šarže*
	GR	*Ημερομηνία λήξης*		GR	*Αριθμός Παρτίδας*
	PT	*Prazo de validade*		PT	*Código do lote*
	HU	*Felhasználható*		HU	*Sarzsszám*
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	IT	*Numero di catalogo*		IT	*Fabbricante*
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	NL	*Catalogus nummer*		NL	*Fabrikant*
	DK	*Katalognummer*		DK	*Producent*
	CZ	*Katalogové číslo*		CZ	*Výrobce*
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	PL	*Numer katalogowy*		PL	*Producent*
	GB	*Contains sufficient for <n> tests*	IVD	GB	*In Vitro Diagnostic Medical Device*
	DE	*Inhalt ausreichend für <n> Prüfungen*		DE	*In-Vitro-Diagnostikum*
	ES	*Contenido suficiente para <n> ensayos*		ES	*Producto sanitario para diagnóstico in vitro*
	IT	*Contenuto sufficiente per "n" saggi*		IT	*Dispositivo medico-diagnostico in vitro*
	FR	*Contenu suffisant pour "n"tests*		FR	*Dispositif médical de diagnostic in vitro*
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	SK	*Obsah postačuje na <n> stanovení*		SK	*Zdravotnícka pomocka in vitro*
	GR	*Περιεχόμενο επαρκές για «ν» εξετάσεις*		GR	*In Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν*
	PT	*Conteúdo suficiente para "n" ensaios*		PT	*Dispositivo médico para diagnóstico in vitro*
	HU	*A doboz tartalma <n> vizsgálat elvégzéséhez elegendő*		HU	*In vitro diagnosztikum*
	SE	*Räcker till "n" antal tester*		SE	*Medicintekniska produkter för in vitro diagnostik*
	PL	*Wystarczy na wykonanie <n> testów*		PL	*Wyrób do diagnistyki In Vitro*
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	FR	*Limites de température*		FR	*Consulter les instructions d'utilisation*
	NL	*Temperatuurlimiet*		NL	*Raadpleeg de gebruiksaanwijzing*
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	CZ	*Teplotní rozmezí od do*		CZ	*Viz návod k použití*
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	SE	*Temperaturbegränsning*		SE	*Se handhavandebeskrivningen*
	PL	*Przestrzegać zakresu temperatury*		PL	*Sprawdź w instrukcji obsługi*

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