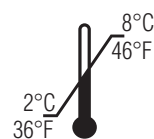


# MICROVUE™ Serum PYD EIA Kit

Bone Health

An enzyme immunoassay for the  
quantitation of  
Pyridinoline Crosslinks in serum

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Not for use in diagnostic procedures.



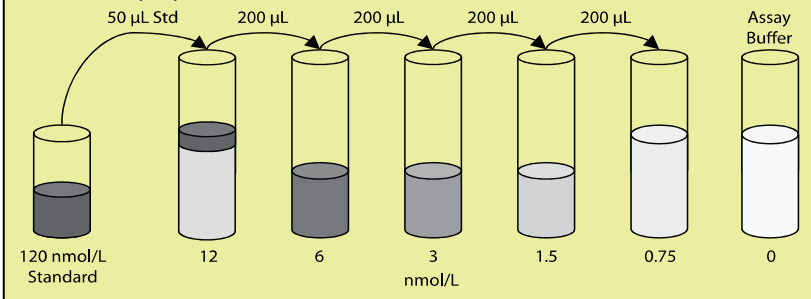
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**REF** 8019

# MicroVue™ Serum PYD EIA Summary

## Reagent and Sample Preparation

- Dilute Controls 1:10 with Assay Buffer (50 µL Control + 450 µL Assay Buffer)
- Prepare 12 nmol/L Standard Solution by diluting the 120 nmol/L PYD Standard 1:10 with Assay Buffer (50 µL PYD Standard + 450 µL Assay Buffer)
- Produce 1:2 dilution series (depicted below) using the 12 nmol/L Standard Solution prepared above (200 µL Working Standard + 200 µL Assay Buffer)



Add ~ 200 µL of each serum sample to 30k MWCO Spinfilter

Centrifuge ~ 30 minutes at 3,000xg to 10,000xg

## Assay Procedure

Pipette 50 µL Reagent 1 into assay wells using multichannel pipette

Pipette 25 µL diluted Standards, diluted Controls, and undiluted filtered Samples into assay wells

Pipette 75 µL cold Pyridinolone antibody into assay wells with sufficient force to ensure adequate mixing.

*Gently tap plate to disperse evenly*

Cover plate and incubate 18–24 hours at 2–8°C in the dark

- Prepare 1X Wash Buffer (Dilute 10X Wash Buffer 1:10 with deionized water)
- Prepare Enzyme Conjugate with 1X Wash Buffer, store at 20–28°C (Add 10 mL 1X Wash Buffer per vial of Conjugate)

*Wash 3 times with 1X Wash Buffer*

Add 150 µL Enzyme Conjugate into assay wells

Incubate 60 ± 5 minutes at 20–28°C

- Prepare Substrate Solution (30 – 60 min before use) Add one Substrate tablet per bottle of Substrate Buffer (Shake vigorously)

*Wash 3 times with 1X Wash Buffer*

Pipette 150 µL Working Substrate Solution

Incubate 40 ± 5 min at 20–28°C

Pipette 100 µL Stop Solution

Read the Optical Density at 405 nm.  
Analyze the assay results using a 4-parameter curve fit  
 $y = (A-D)/(1+(x/C)^B)+D$

## SUMMARY AND EXPLANATION

The MicroVue Serum PYD assay provides a quantitative measure of the excretion of pyridinoline crosslinks in serum. Structural collagens such as types I and II that are present in bone and cartilage, respectively, are crosslinked both within their  $\alpha$ -chains and between adjacent molecules to provide rigidity and strength to the resulting collagen fibril.<sup>1-3</sup> In bone and cartilage, pyridinoline (PYD), a trifunctional pyridinium crosslink, forms between specific hydroxylysine residues within the telopeptide regions of one collagen molecule and within the helical region of an adjacent collagen molecule.<sup>3</sup> When bone or cartilage collagen is degraded, PYD is released into circulation and excreted in the urine. Deoxypyridinoline (DPD), a pyridinium crosslink differing from PYD only by the absence of an hydroxyl group, is essentially bone specific.<sup>1,2,4</sup> The ratio of PYD:DPD in bone of approximately 4:1 is closely concordant with the ratio observed in urine of healthy individuals and those with metabolic bone diseases or osteoarthritis in spite of a much higher PYD:DPD ratio in non-bone tissues.<sup>2,4</sup> This suggests that both crosslinks reflect bone resorption under these conditions. In rheumatoid arthritis, destruction of cartilage and other collagen-containing joint tissue may contribute to increased levels of PYD.<sup>2</sup>

In humans, the total pool of urinary PYD is approximately 45% free, while the remaining fraction is bound to oligopeptides ranging from small linear peptides to very large crosslinked structures in excess of 10,000 Da.<sup>4-7</sup> The proportion of free to total crosslinks appears to be constant in healthy individuals and those with metabolic bone diseases or arthritis conditions,<sup>4,6,8</sup> thus providing the rationale for measuring free PYD. Improvements in immunoassay sensitivity have resulted in the ability to measure free PYD levels in serum, thus permitting a novel method for researching bone and cartilage collagen degradation.<sup>9,10</sup> Since PYD is present and excreted in all mammalian species evaluated, including rodents, dogs, sheep, horses, and nonhuman primates, research applicability of a serum PYD immunoassay extends to animal model studies.

## PRINCIPLE OF THE PROCEDURE

The MicroVue Serum PYD assay is a competitive enzyme immunoassay in a microtiter plate format. PYD in the samples or standards competes with PYD immobilized on the plate for polyclonal rabbit anti-PYD antibody. Bound antibody is detected by goat anti-rabbit antibody conjugated to alkaline phosphatase, and the reaction is detected with pNPP substrate.

## REAGENTS AND MATERIALS PROVIDED

### 96 Assays for the Serum PYD EIA

#### The MicroVue Serum PYD EIA kit contains the following:

- S** Pyridinoline Standard: 120 nmol/L      Part 4552      0.3 mL  
PYD purified from human urine in 10 mmol/L phosphoric acid containing sodium azide (0.05%) as a preservative
- L** Low Controls      Parts 4553      0.3 mL each  
PYD purified from human urine in 10 mmol/L phosphoric acid containing sodium azide (0.05%) as a preservative

<b>H High Controls</b>	<b>Parts 4554</b>	<b>0.3 mL each</b>
PYD purified from human urine in 10 mmol/L phosphoric acid containing sodium azide (0.05%) as a preservative		
<b>1 Coated Strips (Anti-PYD)</b>	<b>Part 4668</b>	<b>12 each</b>
PYD purified from bovine bone adsorbed onto 12 eight-well strips in a resealable foil pouch		
<b>2 Stop Solution</b>	<b>Part 4702</b>	<b>15 mL</b>
0.5N NaOH		
<b>3 10X Wash Buffer</b>	<b>Part 4703</b>	<b>55 mL</b>
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative		
<b>4 Assay Buffer</b>	<b>Part 4704</b>	<b>55 mL</b>
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative		
<b>5 Substrate Buffer</b>	<b>Part 4705</b>	<b>3 x 10 mL</b>
A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative		
<b>6 Substrate Tablets</b>	<b>Part 0012</b>	<b>3 x 20 mg</b>
p-Nitrophenyl phosphate		
<b>7 Enzyme Conjugate</b>	<b>Part 4544</b>	<b>3 each</b>
Lyophilized goat anti-rabbit antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers		
<b>8 Pyridinoline Antibody</b>	<b>Part 4543</b>	<b>9 mL</b>
Rabbit anti-PYD polyclonal antibody containing buffer salts and sodium azide (0.05%) as a preservative		
<b>9 Reagent 1</b>	<b>Part 4744</b>	<b>6 mL</b>
A glycine solution containing indicator dye and ProClin® 300 (0.05%) as a preservative		
<b>Plate Tape Cover</b>	<b>Part 0047</b>	<b>3 each</b>
<b>NanoSpin Filters, 30k MWCO</b>	<b>Part 4935</b>	<b>1 pkg</b>

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### Materials Required But Not Provided

- Timer (60 minute range)
- Glass or plastic tubes for dilution of samples, standards, and controls
- Labware suitable for liquid measurement of 10–300 mL
- Deionized or distilled water
- Wash bottle or other immunoassay washing system
- Adjustable multichannel pipette (8 or 12 channels) or repeating micropipettes (optional)
- Micropipettes and pipette tips to deliver 25–300 µL
- Centrifuge capable of 3,000xg to 10,000xg and able to accommodate spinfilter tubes
- Plate reader capable of reading at 405 nm
- Calculator or other computational method to validate the assay

### WARNINGS AND PRECAUTIONS

1. For Research Use Only. Not for use in diagnostic procedures.
2. Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.

3. Wear suitable protective clothing, gloves, and eye/face protection when handling contents of this kit.
4. Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5. Store assay reagents as indicated.
6. Do not use Coated Strips if pouch is punctured.
7. Test each sample in duplicate.
8. The Stop Solution (4702) is considered corrosive and can cause irritation upon contact. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
9. Sodium azide is used as a preservative. Incidental contact with or ingestion of buffers containing sodium azide can cause irritation to the skin, eyes, or mouth. Only use buffers for intended purposes and avoid contact with acids. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large volume of water to prevent azide build-up.
10. The substrate buffer contains diethanolamine and may cause irritation to the eyes and/or skin with prolonged contact. Contacted areas should be immediately washed with soap and water.
11. The standard and controls contain Pyd purified from human urine and should be treated as potentially biohazardous material.
12. Standard and controls are in 10 mmol/L phosphoric acid. Avoid contact with skin, eyes or clothing. Do not ingest. If contact is made, wash with water. If ingested, call a physician.
13. Use of multichannel pipettes is essential to ensure the timely delivery of reagents. Reagent addition should be uninterrupted.
14. For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
15. Do not use a microassay well for more than one test.
16. Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
17. The Pyridinoline (PYD) standard, controls, coated strips and serum samples are light sensitive. Avoid prolonged exposure to light, especially direct or indirect sunlight. Store reagents in the dark when not in use. Samples and reagents are not significantly affected by normal, artificial laboratory lighting when handled as directed in the *ASSAY PROCEDURE*.
18. Do not allow microassay wells to dry once the assay has begun.
19. When adding or removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
20. A wash bottle or automated filling device should be used to wash the plate (*ASSAY PROCEDURE*, Step 6). For best results, do not use a multichannel pipette to wash the microassay plate.
21. Perform this assay with any validated washing method.
22. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.

## REAGENT PREPARATION

### Wash Buffer

See Procedural Note under *ASSAY PROCEDURE*.

Prepare required amount of 1X Wash Buffer (see table in *ASSAY PROCEDURE* section) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at 20–28°C. Use 1X Wash Buffer within 21 days of preparation.

### Enzyme Conjugate

Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table in *ASSAY PROCEDURE* section) with 10 mL 1X Wash Buffer. Store reconstituted Enzyme Conjugate at 20–28°C until use.

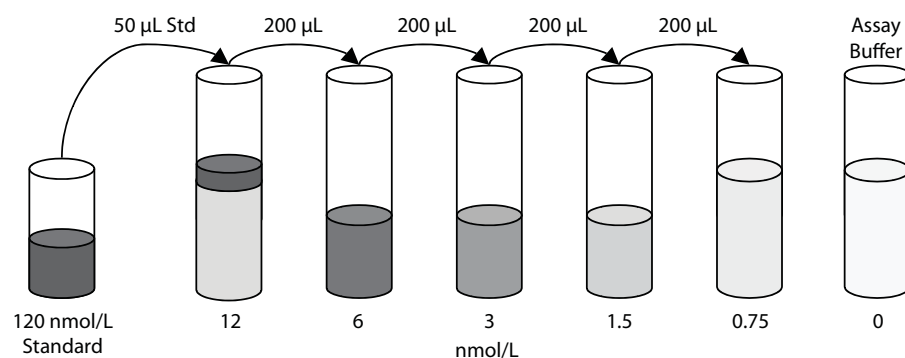
### Prepared Substrate Solution

Bring the Substrate Buffer to 20–28°C before beginning the assay. (Two hours to overnight recommended.) Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20–28°C Substrate Buffer (see table in *ASSAY PROCEDURE* section). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

### PYD Standard

Prepare 12 nmol/L Standard Solution by diluting the 120 nmol/L PYD Standard 1:10 with Assay Buffer (50  $\mu$ L PYD Standard + 450  $\mu$ L Assay Buffer).

Use the 1:10 12 nmol/L Standard Solution prepared above to produce the 1:2 dilution series depicted below (200  $\mu$ L Working Standard + 200  $\mu$ L Assay Buffer). Mix each tube thoroughly before the next transfer. The 1:10 Standard Solution serves as the high standard (12 nmol/L) and Assay Buffer serves as the zero standard (0 nmol/L). A fresh set of standards should be prepared each day.



## CONTROLS

Dilute Controls 1:10 with Assay Buffer (50  $\mu$ L Control + 450  $\mu$ L Assay Buffer).

## INDICATIONS OF REAGENT INSTABILITY / DETERIORATION

Cloudiness, discoloration, or offensive odor may indicate instability or deterioration of kit reagents. If this occurs, the reagent should be discarded.

## STORAGE

Store the unopened kit at 2–8°C.

All reagents must be brought to 20–28°C before use.

Place all unused microassay strips into the storage bag, reseal the bag, and store at 2–8°C.

## SPECIMEN COLLECTION AND STORAGE

Handle and dispose of all specimens using Universal Precautions.

Collect the serum using standard venipuncture technique.

Specimens should be processed to avoid hemolysis. Keep the serum sample refrigerated (2–8°C) for storage of less than 4 days, or freeze the sample at -20°C for longer storage. Do not subject the samples to more than 3 freeze/thaw cycles. Avoid prolonged exposure to light, especially direct sunlight. Samples are not affected by normal, artificial laboratory lighting.

## SAMPLE PREPARATION

Filter serum samples by adding approximately 200 µL of each serum to a 30k MWCO Spinfilter (1 basket and 1 receptacle per spinfilter).

Centrifuge approximately 30 minutes at 3,000xg to 10,000xg.

Filtrate should be colorless. Repeat filtration if filtrate is yellow.

Protect filtrate from prolonged (more than 1 hour) exposure to light.

Samples should be prepared for same day use.

## ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See *WARNINGS AND PRECAUTIONS* and *REAGENT PREPARATION*.

Determine amount of each reagent required for the number of strips to be used.

# of Strips	4	6	8	12
# of Samples (tested in duplicate)	8	16	24	40
Enzyme Conjugate (vial)	1	1	2*	2*
Substrate (bottle)	1	1	2*	2*
1X Wash Buffer (mL)	100	150	200	300

When more than one bottle or vial is to be used, combine the contents and mix prior to use.

1. Prepare Standards, Controls, samples and reagents as instructed.
2. Allow pouch of Coated Strips to equilibrate to room temperature before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table). Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.
3. Add 50 µL Reagent 1 to each well of the Coated Strips using a multichannel pipette.
4. Add 25 µL diluted Standard, Control, or undiluted filtered sample to each well of the Coated Strips. This step should be completed within 20 minutes.
5. Add 75 µL of cold Pyridinoline antibody to each well. Dispense Pyridinoline antibody with sufficient force to ensure adequate mixing. Tap Stripwell Frame several times. Cover plate with Tape Cover provided. Incubate overnight (18–24 hours) at 2–8°C. This incubation should be carried out in the dark.

6. Manually invert/empty strips. Add at least 250 µL of prepared 1X Wash Buffer to each well and manually invert/empty strips. Repeat two more times for a total of three washes. Vigorously blot the strips dry on paper towels after the last wash.
7. Add 150 µL of room temperature reconstituted Enzyme Conjugate to each well (add 10 mL of 1X Wash Buffer to each required vial). Incubate for 60 ± 5 minutes at 20–28°C.
8. Manually invert/empty strips. Add at least 250 µL of 1X Wash Buffer to each well and manually invert/ empty strips. Repeat two more times for a total of three washes. Vigorously blot the strips dry on paper towels after the last wash. While the strips are inverted, carefully wipe bottom of strips with a lint-free paper towel to ensure that the bottoms of the strips are clean.
9. Add 150 µL of Working Substrate Solution to each well. Incubate for 40 ± 5 minutes at 20–28°C. **NOTE:** If room temperature cannot be maintained between 20–28°C and an absorbance of > 2.0 is not compatible with your plate reader, monitor the development of substrate in the zero standard wells; stop the reaction when the optical density reaches 1.2–1.5; then read the strip(s).
10. Add 100 µL of Stop Solution to each well to stop the reaction. Add Stop Solution in the same pattern and time intervals as the Substrate Solution addition.
11. Read the optical density at 405 nm. Assure that no large bubbles are present in wells and that the bottoms of the strips are clean. Strips should be read within **15 minutes** of Stop Solution addition.
12. Analyze Serum PYD assay results using quantitation software with a 4 parameter calibration curve fitting equation:
 
$$y = (A-D)/(1+(x/C)^B)+D$$
13. Determine concentration of samples and Controls from the standard curve.
  - a. Dilute samples greater than 12 nmol/L in Assay Buffer and retest. Include the dilution factor in the final calculation.
  - b. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

## QUALITY CONTROL

The Certificate of Analysis included in this kit is lot specific and is to be used to verify that the results obtained by your laboratory are similar to those obtained at Quidel Corporation. The optical density values are provided and are to be used as a guideline only. The results obtained by your laboratory may differ.

Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should be considered questionable and the samples should be repeated.

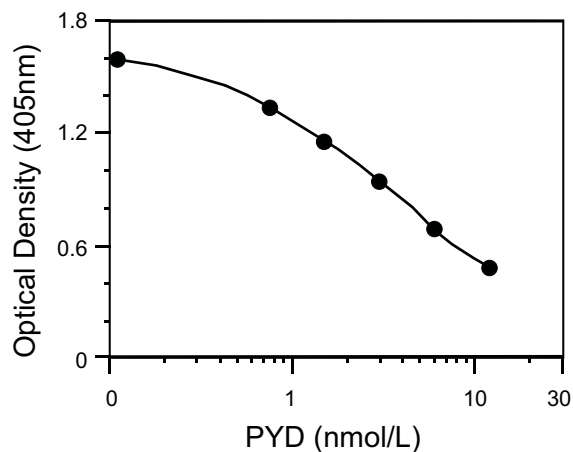
If the optical density of the zero standard (Assay Buffer) is less than 0.8, the results should be considered questionable, and the samples should be repeated.

## INTERPRETATION OF RESULTS

Sample results are expressed as nmol/L and do not need to be corrected for dilution.

### Representative Standard Curve

Standard PYD levels: 0, 0.75, 1.5, 3, 6, 12 nmol/L



### EXAMPLE VALUES

In our testing of 59 adults aged 25–55 years, values obtained from the MicroVue Serum PYD kit had a mean value of 1.57 nmol/L, with a range of 1.090–2.792 nmol/L. Each laboratory should establish its own reference range.

### PERFORMANCE OF THE TEST

#### Specificity of the Antibody

The Pyridinoline antibody demonstrates selective, high affinity for free pyridinoline and negligible binding to DPD, and PYD and DPD peptides.

	% Reactivity
Free PYD	100%
Free DPD	0%
PYD/DPD peptides $\geq$ 1000 MW	< 2.5%
L-Arginine	0.0004%
L-Glutamine	0.000009%

Physiological levels of arginine and glutamine contribute to the measured PYD value (equivalent to approximately 0.5 nmol/L). Physiological levels of other amino acids are undetectable.

#### Reactivity to Pyridinoline in Animal Sera

The Pyridinoline antibody demonstrates reactivity to free PYD from the following animal species: mouse, rat, guinea pig, dog, cow, horse and primate (monkey, baboon).

(Rabbit reacts but is not compatible in this assay due to possible interference of endogenous rabbit antibodies.)

#### Limits of Detection

The minimum detection limit of the Serum PYD Assay is 0.4 nmol/L, determined by the upper 3 SD limit in a zero standard study.

## Precision

Within-run and between-run precision were determined by assaying four serum samples.

PYD nmol/L	Within-run <sup>1</sup> CV%	Between-run <sup>2</sup> CV%
1.04	14.8	11.6
2.26	8.3	8.7
4.63	6.3	9.5
8.13	8.0	10.4

<sup>1</sup> 12 replicates      <sup>2</sup> 20 runs

## Recovery - Linearity

Linearity was determined by serially diluting samples of all listed species and comparing observed values with expected values.

Average recovery was 107%.

Absolute range: 93–122%.

## Recovery - Spike Recovery

Spike recovery was determined by adding known quantities of purified PYD to serum samples of all listed species.

Average recovery was 100%.

Absolute range 91–109%.

## Interfering Substances

The following substances were tested at the specified concentrations, and were not found to interfere with the assay.

Substance	Concentration
Bilirubin, conjugated	40 mg/dL
Cholesterol	500 mg/dL
Hemoglobin	500 mg/dL
Protein, Total	12 g/dL
Protein, Albumin	6 g/dL
Protein, g-Globulin	6 g/dL
Triglycerides (lipemia)	3000 mg/dL

## ASSISTANCE

To place an order or for technical assistance, please contact a Quidel Representative at 800-524-6318 or 408-616-4301, Monday through Friday, between 8:00 a.m. and 5:00 p.m., Pacific Time. Orders may also be placed by fax at 408-616-4310.

For services outside the U.S., please contact your local distributor. Additional information about Quidel and Quidel's products and distributors can be found on our website at [www.quidel.com](http://www.quidel.com).

## REFERENCES

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Manufacturer



Catalog Number



Consult Instructions for Use



Temperature Limitation



Contents / Contains



Instructions for Use on CDROM



Contains sufficient for <n> tests



Biological Risks

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**REF** 8019 – MicroVue™ Serum PYD Enzyme Immunoassay Kit