

# METRA™ CICP EIA kit

96 Assays for the C-terminal Propeptide  
of Type I Collagen in Serum

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

Store at 2-8°C

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Made in USA

0201E Rev. 10/01

Catalog number 8003

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Read the entire product insert thoroughly before beginning the assay. The Metra™ CICP kit should be stored at 2-8°C until use.

## SUMMARY AND EXPLANATION

Collagen, the triple-helical molecule which forms the fibrous framework of all connective tissues, is synthesized as procollagen, a larger precursor molecule. Procollagen consists of mature collagen with extension peptides at both the amino and carboxy termini. These extension peptides, or propeptides, are cleaved from the collagen molecule by specific proteases prior to incorporation of collagen into a growing collagen fibril. The release of these peptides into the circulation provides a stoichiometric representation of the production of collagen.

## PRINCIPLE OF THE PROCEDURE

The Metra™ CICP assay is a sandwich enzyme immunoassay in a microtiter plate format utilizing a monoclonal anti-CICP antibody coated on the plate, a rabbit anti-CICP antiserum, a goat anti-rabbit alkaline phosphatase conjugate, and a pNPP substrate to quantify CICP in human serum.

## REAGENTS AND MATERIALS

**Metra CICP part number 8003 contains the following:**

**Substrate Tablets**                      **Part 0012**                      **3 each**  
p-Nitrophenyl phosphate (20 mg each).

**CICP Standards:**                      **Parts 4138 - 4143**                      **0.75 mL each**  
**(0, 1, 2, 5, 20, 80 ng/mL)**

CICP purified from human fibroblast cells in a buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative.

**Low/High Controls**                      **Parts 4144, 4145**                      **0.75 mL each**  
CICP purified from human fibroblast cells in a buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative.

**Rabbit anti-CICP**                      **Part 4148**                      **14 mL**

Rabbit polyclonal anti-CICP antibody in a buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative.

**Enzyme Conjugate**                      **Part 4149**                      **3 each**

Lyophilized goat anti-rabbit IgG antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers.

**Assay Buffer**                      **Part 4150**                      **20 mL**

A buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative.

**Coated Strips**                      **Part 4672**                      **1 each**

Purified murine monoclonal anti-CICP antibody adsorbed onto stripwells.

**Stop Solution**                      **Part 4702**                      **15 mL**

1N NaOH

**10X Wash Buffer**                      **Part 4703**                      **55 mL**

Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.

**Substrate Buffer**                      **Part 4705**                      **10 mL**

A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative.

## QUICK GUIDE TO ASSAY STEPS

1. Dilute samples 1:12.
2. Add 100 µL Standards, Controls and diluted samples.
3. Incubate 120 ± 5 minutes at room temperature (18-25°C).
4. Wash 3 times with 1X Wash Buffer.
5. Add 100 µL Rabbit anti-CICP.
6. Incubate 45-50 minutes at room temperature (18-25°C).
7. Wash 3 times with 1X Wash Buffer.
8. Add 100 µL Enzyme Conjugate.
9. Incubate 45-50 minutes at room temperature (18-25°C).
10. Wash 3 times with 1X Wash Buffer.
11. Add 100 µL Working Substrate Solution.
12. Incubate 30-35 minutes at room temperature (18-25°C).
13. Add 50 µL Stop Solution and read OD at 405 nm.

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## WARNINGS

1. Serum samples should be treated as potentially biohazardous material.
2. 1N NaOH is poisonous and can cause severe burns. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
3. Sodium azide is used as a preservative. It may be fatal if swallowed or adsorbed through the skin. Do not mix 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.
4. Test kits and components should be disposed of in a manner consistent with relevant regulations.

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## PRECAUTIONS

1. Use assay reagents as an integral unit prior to the expiration date indicated on the package label.
2. Store assay reagents as indicated.
3. Do not use Coated Strips if pouch is punctured.
4. Samples greater than 80 ng/mL should be diluted in Assay Buffer and retested.
5. Test each sample in duplicate.
6. Perform a standard curve with each assay.
7. Use a 4-parameter calibration curve fit for accurate results.  
Equation:  $y = (A-D)/(1+(x/C)^B)+D$
8. Perform this assay with any validated washing method.
9. 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 OD values provided 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.

10. If the OD of the Standard F is less than 0.8, the results should be considered questionable and the samples should be repeated.
11. If room temperature cannot be maintained between 18-25°C and an absorbance of > 2.0 is not compatible with your plate reader, monitor the development of substrate in the Standard F wells; stop the reaction when the OD reaches 1.2-1.5; then read the strip(s).
12. Use of multichannel pipettes or repeat pipetors is recommended to ensure timely delivery of reagents.
13. For accurate measurement of samples, the addition of samples and standards must be precise. Pipet carefully using only calibrated equipment.
14. Standards, Controls and samples must be added to the strip wells within 30 minutes.

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## REAGENT PREPARATION AND STORAGE

**Bring reagents to room temperature (18-25°C) before use. It is recommended that samples be assayed in duplicate.**

1. Coated Strips  
Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table in Assay Procedure Section). Ensure that the pouch containing any unused strips is completely resealed.
2. Wash Buffer  
Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at room temperature (18-25°C). Use 1X Wash Buffer within 24 hours of preparation.
3. Enzyme Conjugate  
Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of 1X Wash Buffer. Allow pellet to completely dissolve. Discard remaining Enzyme Conjugate after use.
4. Working Substrate Solution  
Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix. Discard remaining Working Substrate Solution after use.

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## SPECIMEN COLLECTION AND STORAGE

Collect the serum specimens using standard venipuncture technique, without anti-coagulants, and in such a way to avoid hemolysis. Allow the blood to clot and separate the serum by centrifugation. Store serum refrigerated (2-8°C) for storage of less than 5 days, or frozen at ≤ -20°C for longer storage. Do not subject the samples to more than 3 freeze/thaw cycles.

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## METRA CICIP ASSAY PROCEDURE

### Metra CICIP part number 8003

<u>Kit Contents</u>	<u>Qty/Vol</u>	<u>Part</u>
Substrate Tablets	3 each	0012
Standard A (CICP 0 ng/mL)	0.75 mL	4138
Standard B (CICP 1 ng/mL)	0.75 mL	4139
Standard C (CICP 2 ng/mL)	0.75 mL	4140
Standard D (CICP 5 ng/mL)	0.75 mL	4141
Standard E (CICP 20 ng/mL)	0.75 mL	4142
Standard F (CICP 80 ng/mL)	0.75 mL	4143
Control, Low	0.75 mL	4144
Control, High	0.75 mL	4145
Rabbit anti-CICP	14 mL	4148
Enzyme Conjugate (lyophilized)	3 each	4149
Assay Buffer	20 mL	4150
Stop Solution	15 mL	4702
10X Wash Buffer	55 mL	4703
Substrate Buffer (3)	10 mL	4705
Coated Strips (12)	1 each	4672

### Materials Required BUT NOT Provided

Micropipettes to deliver 25-300 µL  
Labware suitable for liquid measurement of 7-300 mL  
Tubes for dilution of samples  
Container for wash buffer dilution  
Deionized or distilled water  
Plate reader capable of reading at 405 nm  
4-parameter calibration curve fitting software

## METRA CICIP ASSAY PROCEDURE (CONT.)

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 Buffer (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 of each vial, and mix prior to use.

### Sample Dilution/Incubation

1. Dilute serum samples 1:12 with Assay Buffer (e.g. 25  $\mu$ L serum + 275  $\mu$ L Assay Buffer).
2. Allow pouch of Coated Strips to equilibrate to room temperature (18-25°C) before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table). Ensure that the pouch containing unused strips is completely resealed and contains desiccant.
3. Place desired number of Coated Strips in Stripwell Frame just prior to use. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.
4. Add 100  $\mu$ L Standard, Control, or diluted serum sample to each well of the Coated Strips. This step should be completed within 30 minutes.
5. Incubate 120  $\pm$  5 minutes at room temperature (18-25°C).

### Antibody Incubation

1. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at room temperature (18-25°C). Use 1X Wash Buffer within 24 hours of preparation.
2. Manually invert/empty strips. Add at least 300  $\mu$ L 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.
3. Add 100  $\mu$ L of Rabbit anti-CICIP to each well.
4. Incubate 45-50 minutes at room temperature (18-25°C).

### Enzyme Conjugate Incubation

1. Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of 1X Wash Buffer. Mix thoroughly.
2. Manually invert/empty strips. Add at least 300  $\mu$ L 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.
3. Add 100  $\mu$ L of reconstituted Enzyme Conjugate to each well.
4. Incubate 45-50 minutes at room temperature (18-25°C).

## METRA CICIP ASSAY PROCEDURE (CONT.)

### Substrate Incubation

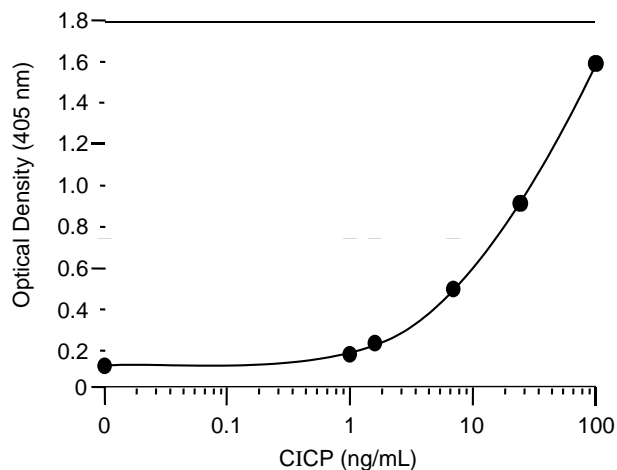
1. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of room temperature Substrate Buffer (see table). Allow 30-60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.
2. Manually invert/empty strips. Add at least 300  $\mu$ L 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.
3. Add 100  $\mu$ L of Working Substrate Solution to each well.
4. Incubate for 30-35 minutes at room temperature (18-25°C). Read Precaution #11 if room temperature cannot be maintained in this range.

### Stop/Read

1. Add 50  $\mu$ L of Stop Solution to each well to stop the reaction.
2. Read the Optical Density (OD) at 405 nm. Assure that no large bubbles are present in wells and that the bottom of the strips are clean. Read strips within **15 minutes** of Stop Solution addition.
3. Use quantitation software with a 4-parameter calibration curve fitting equation to analyze the Metra CICIP assay results.
4. Determine concentration of samples and Controls from the standard curve.
5. Control values should be within the range specified in the Certificate of Analysis supplied with the kit.

### Representative Standard Curve

Standard CICIP levels: 0, 1, 2, 5, 20, 80 ng/mL



## INTERPRETATION OF RESULTS

Sample results **must** be corrected for the dilution made. If the sample was diluted 1:12, **multiply the ng/mL value by 12** for the final result of serum CICIP in ng/mL.

## METRA CICP EXPECTED VALUES

Preliminary CICP reference ranges have been established for healthy adult males (n=53), healthy adult premenopausal females (n=226) over 25 years of age, and children. For the purposes of establishing reference ranges, healthy adult subjects were defined as:

- Basically healthy, no bone, endocrine or chronic disorders
- Regular menstrual cycles (females)
- Not pregnant or breast feeding (females)
- Not currently taking any medication known to influence bone metabolism (e.g., anticonvulsants, bisphosphonates, calcitonin, corticosteroids, GnRH analogs, heparin, hormone replacement therapy, and thyroid medication)

Values may be influenced by such factors as growth, low estrogen production, low calcium intake or low physical activity. Estrogen deficiency in postmenopausal women can result in elevated bone turnover, and thus collagen production. It is suggested that the premenopausal reference range be used to interpret results in postmenopausal women. Each laboratory should establish its own reference range.

### Adults

Sex	Range
Females	69-147 ng/mL
Males	76-163 ng/mL

### Children

Females:		CICP (ng/mL)		
Age (years)	N	Mean	Min.	Max.
4-10	46	306	136	527
11-13	150	346	118	961
14-18	41	226	110	443

Males:		CICP (ng/mL)		
Age (years)	N	Mean	Min.	Max.
4-10	23	299	149	426
11-13	55	398	113	943
14-18	55	378	130	66

## PERFORMANCE CHARACTERISTICS

### Specificity

The anti-CICP antibodies have been raised against CICP derived from human fibroblast cells in culture. The antibodies demonstrate 100% crossreactivity with CICP in human serum.

### Sensitivity

The minimum detection limit of the Metra CICP Assay is 0.2 ng/mL, determined by the upper 3 SD limit in a zero standard study.

### Precision

Within-run and between-run precision were determined by assaying three serum samples in multiple runs. Typical results are provided below.

Prolagen-C CICP (ng/mL)	Within-run <sup>1</sup> C.V. (%)	Between-run <sup>2</sup> C.V. (%)
80.8	6.8	7.0
98.1	5.5	7.2
296.7	6.6	5.0

<sup>1</sup> n = 20      <sup>2</sup> n = 3 in 3 runs

## PERFORMANCE CHARACTERISTICS (CONT.)

### Recovery - Linearity

Linearity was determined by serially diluting samples and comparing observed values with expected values. Typical results are provided below.

Sample	Dilution Factor	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
1	1:12	6.90	-	-
	1:24	3.50	3.45	101
	1:48	1.74	1.72	101
2	1:12	13.26	-	-
	1:24	6.56	6.63	99
	1:48	3.49	3.32	105
3	1:12	20.88	-	-
	1:24	10.43	10.44	100
	1:48	5.57	5.22	107

### Recovery - Spike Recovery

Spike recovery was determined by adding known quantities of purified CICP to serum samples with different levels of endogenous CICP. Typical results are provided below.

Sample	Endogenous (ng/mL)	Added (ng/mL)	Observed (ng/mL)	Recovery (%)
1	9.09	13.24	22.28	100
		31.77	45.96	102
2	10.34	13.10	23.00	97
		32.71	43.05	96
3	12.43	13.24	22.28	100
		31.77	41.55	102

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