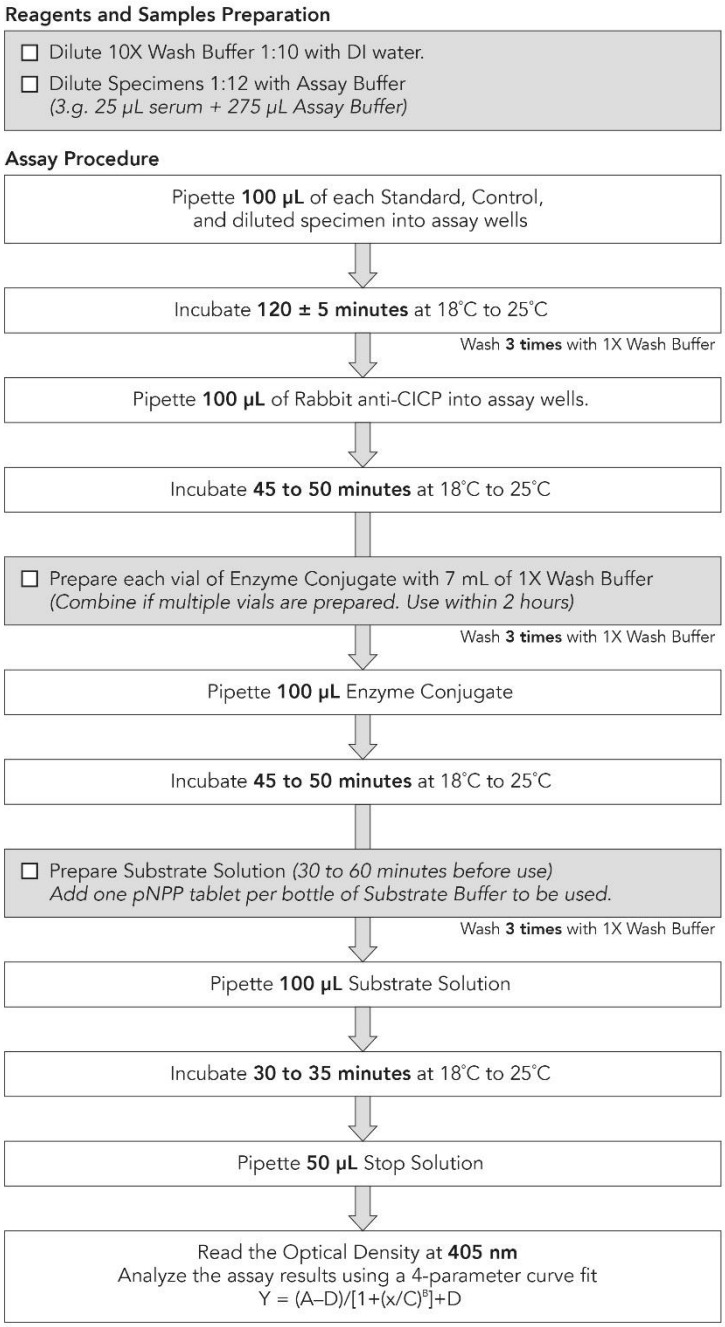
QUIDEL

MicroVue™ Bone CICP EIA

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

SUMMARY



INTENDED USE

The MicroVue CICP Assay is an enzyme immunoassay to quantitatively measure C-terminal Propeptide of Type I Collagen (CICP).

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.

The MicroVue CICP EIA Kit provides a quantitative method for determining levels of CICP (C-Terminal of Type I Collagen) in serum. Levels of CICP are indicative of collagen production *in vivo.* As the primary organic constituent of bone, Type I Collagen levels have been linked to bone growth and formation. Elevated levels of CICP have been shown in diseases associated with high levels of bone turnover, including Paget's disease of the bone, hyperthyroidism, primary hyperparathyroidism and renal osteodystrophy. In some cases, elevated levels of CICP have also been documented in early menopause. Low levels of CICP have been shown in growth hormone-deficient children.

PRINCIPLE OF THE PROCEDURE

The MicroVue 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.

REAGENTS AND MATERIALS PROVIDED

96 Assays for the C-terminal Propeptide of Type I Collagen in Serum
MicroVue CICP EIA kit contains the following:

A **CICP Standards:** **Parts 4138-4143** **0.75 mL each**
B Standards A-F, ready to use, concentration see Certificate of Analysis. CICP purified from human
C fibroblast cells in a buffered solution containing nonionic detergent, stabilizer, and sodium azide
D (0.05%) as a preservative
E
F

L **Low Control** **Part 4144** **0.75 mL**
CICP purified from human fibroblast cells in a buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative

H **High Control** **Part 4145** **0.75 mL**
CICP purified from human fibroblast cells in a buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative

❶ **Coated Strips** **Part 4672** **12 each**
Purified murine monoclonal anti-CICP antibody adsorbed onto stripwells

❷ **Stop Solution** **Part 4702** **15 mL**
0.5 N NaOH

❸ **10X Wash Buffer** **Part 4703** **55 mL**
Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative

❹ **Assay Buffer** **Part 4150** **20 mL**
A buffered solution containing nonionic detergent, stabilizer, and sodium azide (0.05%) as a preservative

❺ **Substrate Buffer** **Part 4705** **10 mL, 3 each**
A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative

❻ **Substrate Tablets** **Part 0012** **20 mg, 3 each**
p-Nitrophenyl phosphate

❼ **Enzyme Conjugate** **Part 4149** **3 vials**
Lyophilized goat anti-rabbit IgG antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers

❽ **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

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 25 µL to 300 µL
- Labware suitable for liquid measurement of 7 ml to 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

WARNINGS AND PRECAUTIONS

- For Research Use Only. Not for use in diagnostic procedures (U.S. only).
- Treat specimen samples as potentially biohazardous material. Follow Universal Precautions when handling contents of this kit and any patient samples.
- Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
- Store assay reagents as indicated.
- Do not use Coated Strips if pouch is punctured.
- The Stop Solution (0.5 N NaOH) is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, wash with water. If ingested, call a physician.
- 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.
- 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.
- Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipette carefully using only calibrated equipment.
- Proper collection and storage of test specimens are essential for accurate results (see *SPECIMEN COLLECTION AND STORAGE*).
- Avoid microbial or cross-contamination of specimens or reagents.
- Test each sample in duplicate.

- Do not use a microassay well for more than one test.
- Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
- Do not allow microassay wells to dry once the assay has begun.
- When [adding or] removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
- Heat inactivated, hyperlipemic, or contaminated specimens may give erroneous results.
- To avoid aerosol formation during washing, use an apparatus to aspirate the wash fluid into a bottle containing household bleach.
- This assay may be performed with any validated washing method.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

Store kit at 2°C to 8°C. Store unused reagents at 2°C to 8°C. Store 1X Wash Buffer (10X diluted) at 18°C to 28°C.

SPECIMEN COLLECTION AND PREPARATION

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°C to 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.

REAGENT PREPARATION

Equilibrate reagents to 18°C to 25°C prior to use.

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.

Wash Buffer

Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at 18°C to 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) with 7 mL of 1X Wash Buffer. Allow the pellet to completely dissolve. Discard remaining Enzyme Conjugate after use.

Working Substrate Solution

Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 18°C to 25°C Substrate Buffer (see table). Allow 30 to 60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix. Discard remaining Working Substrate Solution after use.

ASSAY PROCEDURE

Read entire product insert before beginning the assay.

See REAGENT PREPARATION before proceeding.

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 and mix prior to use

Sample Dilution/Incubation

1. Dilute serum samples 1:12 with Assay Buffer (e.g. 25 µL serum + 275 µL Assay Buffer).
2. Allow pouch of Coated Strips to equilibrate to 18°C to 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 µL Standards, Controls, or diluted serum sample to each well of the Coated Strips. This step should be completed within 30 minutes.
5. Incubate 120 ± 5 minutes at 18°C to 25°C.

Washing Step (1)

6. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer concentrate 1:10 with deionized water. Store at 18°C to 28°C. Use 1X Wash Buffer within 21 days of preparation.
7. Manually invert/empty strips. Add at least 300 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.

Antibody Incubation

8. Add 100 µL of Rabbit anti-CICP to each well.
9. Incubate 45-50 minutes at 18°C to 25°C.
10. While incubating, prepare Enzyme Conjugate. Reconstitute each required vial of Enzyme Conjugate (see table) with 7 mL of 1X Wash Buffer. Mix thoroughly. Use within 2 hours.

Washing Step (2)

11. Manually invert/empty strips. Add at least 300 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.

Enzyme Conjugate Incubation

12. Add 100 µL of reconstituted Enzyme Conjugate to each well.
13. Incubate 45 to 50 minutes at 18°C to 25°C.

14. While incubating, prepare Working Substrate Solution. Put one Substrate Tablet into each required bottle of 18°C to 25°C Substrate Buffer (see table). Allow 30 to 60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix. Use within 1 hour.

Washing Step (3)

15. Manually invert/empty strips. Add at least 300 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.

Substrate Incubation

16. Add 100 µL of Working Substrate Solution to each well.
17. Incubate for 30 to 35 minutes at 18°C to 25°C. If room temperature cannot be maintained between 18°C to 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 optical density reaches 1.2 to 1.5; then read the strip(s).

Stop/Read

18. Add 50 µL of Stop Solution to each well to stop the reaction.
19. 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. Read strips within 15 minutes of Stop Solution addition.
20. Use quantitation software with a 4-parameter calibration curve fitting equation (see below) to analyze the MicroVue CICP assay results.

    Equation: $y = (A–D)/(1+(x/C)^{B})+D$

21. Determine concentration of samples and Controls from the standard curve.
    Dilute samples greater than 80 ng/mL in Assay Buffer and retest. 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.

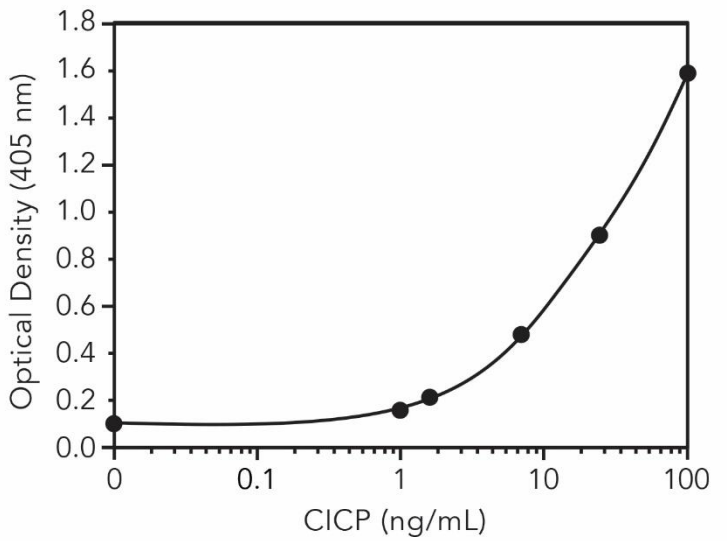
Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should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

If the optical density of the MicroVue CICP Standard F is less than 0.8, the results should be considered questionable, and the samples should be repeated.

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 CICP in ng/mL.

Representative Standard Curve



PERFORMANCE OF THE TEST

Specificity of the Antibodies

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

Limits of Detection

The minimum analytical detection limit of the MicroVue 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. Typical results are provided below.

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

[1]n = 20 replicates [2]n = 3 in 3 runs

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	13.00	97
		32.71	43.05	96
3	12.43	13.24	22.28	100
		31.77	41.55	102

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website **quidel.com**.

REFERENCES

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REF 8005 – MicroVue CICP EIA Kit

RUO

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PI8005000EN00 (10/17)

GLOSSARY

REF Catalogue number	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Consult e-labeling instructions for use
RUO For Research use only	 Contains sufficient for 96 determinations
CONT Contents/Contains	CONTROL Control