

# METRA™ Osteocalcin EIA kit

96 Assays for Osteocalcin

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

Store at 2–8°C

**Quidel Corporation**  
Worldwide Headquarters  
10165 McKellar Court  
San Diego, CA 92121 USA  
[www.quidel.com](http://www.quidel.com)

For other locations log onto our website.

Made in USA

0125K (10/01)

Catalog number 8002

**QUIDEL®**

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

## QUICK GUIDE TO ASSAY STEPS

1. Add 25µL Standards, Controls, and samples
2. Add 125µL anti-Osteocalcin antibody
3. Incubate 2 hours ± 10 minutes at room temperature
4. Wash 3 times with 1X Wash Buffer
5. Add 150µL reconstituted Enzyme Conjugate
6. Incubate 60 ± 5 minutes at room temperature
7. Wash 3 times with 1X Wash Buffer
8. Add 150µL Working Substrate Solution
9. Incubate 35–40 minutes at room temperature
10. Add 50µL Stop Solution and read OD at 405nm

## INTENDED USE

The Metra™ Osteocalcin immunoassay quantitatively measures intact (*de novo*) osteocalcin in serum. Intact osteocalcin may be useful as a biochemical indicator of bone turnover.

## SUMMARY AND EXPLANATION

Osteocalcin (OC) or BGP (bone gla protein) is found exclusively in bone tissue. It is a 5800 molecular weight extrahepatic vitamin K dependent protein produced by osteoblasts. It contains three gamma-carboxyglutamic acid residues which are thought to be involved in calcium ion and hydroxyapatite binding. It accounts for 10–20% of the noncollagenous protein in bone. While the *in vivo* function of osteocalcin is unknown, its affinity for bone mineral constituents implies a role in bone formation

## PRINCIPLE OF THE PROCEDURE

The Metra™ Osteocalcin assay is a competitive immunoassay. The assay uses osteocalcin coated strips, a mouse anti-osteocalcin antibody, an anti-mouse IgG-alkaline phosphatase conjugate and a pNPP substrate to quantify osteocalcin in serum.

## REAGENTS AND MATERIALS

The Metra™ Osteocalcin EIA Kit, part number 8002, contains the following:

<b>Substrate Tablets</b> p-Nitrophenyl phosphate (20mg each).	<b>Part 0012</b>	<b>3 each</b>
<b>Anti-Osteocalcin</b> Purified murine monoclonal anti-osteocalcin antibody in a buffered solution containing nonionic detergent, stabilizers, and sodium azide (0.05%) as a preservative.	<b>Part 4089</b>	<b>15 mL</b>
<b>Osteocalcin Standards:</b> (0, 2, 4, 8, 16, 32 ng/mL) Lyophilized osteocalcin, purified from human bone, containing buffer salts and stabilizers.	<b>Parts 4168–4173</b>	<b>1 each</b>
<b>Low/High Controls</b> Lyophilized osteocalcin, purified from human bone, containing buffer salts and stabilizers.	<b>Parts 4174, 4175</b>	<b>1 each</b>
<b>Enzyme Conjugate</b> Lyophilized goat anti-mouse IgG antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers.	<b>Part 4180</b>	<b>3 each</b>
<b>Coated Strips</b> Osteocalcin purified from human bone adsorbed onto stripwells.	<b>Part 4670</b>	<b>12 each</b>
<b>Stop Solution</b> 1N NaOH	<b>Part 4702</b>	<b>10 mL</b>
<b>10X Wash Buffer</b> Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative.	<b>Part 4703</b>	<b>55 mL</b>
<b>Substrate Buffer</b> A diethanolamine and magnesium chloride solution containing sodium azide (0.05%) as a preservative.	<b>Part 4705</b>	<b>3 x 10 mL each</b>

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## WARNINGS AND PRECAUTIONS

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

1. All 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 absorbed 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.
5. All reagents supplied should be used as an integral unit prior to the expiration date indicated on the package label.
6. Assay reagents should be stored as indicated.
7. Do not use Coated Strips if pouch is punctured.
8. Each sample should be tested in duplicate.
9. A standard curve must be performed with each assay.
10. A 4-parameter calibration curve fit must be used for accurate results. Equation:  $y = (A-D)/(1+(x/C)^B)+D$
11. Samples greater than 32ng/mL should be diluted in 1X Wash Buffer and retested.
12. 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 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.
13. If the OD of the Metra™ Osteocalcin Standard A is less than 0.8, the results should be considered questionable and the samples should be repeated.
14. If room temperature cannot be maintained between 20-25°C and an absorbance of > 2.0 is not compatible with your plate reader, monitor the development of substrate in the Standard A wells; stop the reaction when the OD reaches 1.5; then read the strips.
15. Use of multichannel pipets or repeat pipetors is recommended to ensure the timely delivery of reagents.
16. For accurate measurement of samples, the addition of samples and standards must be precise. Pipet carefully using only calibrated equipment.
17. Adequate wash buffer volume is critical; pipet at least 300µL per well in the wash steps. This assay may be performed with any validated washing method.

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## WARNINGS AND PRECAUTIONS (CONT.)

18. The Sample/Anti-Osteocalcin Incubation should be performed at the same temperature each time the assay is run (within ±1°C). If consistent room temperature cannot be maintained, use of an incubator is recommended.

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

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## REAGENT STORAGE

1. Store the kit at 2-8°C.
2. All reagents must be brought to room temperature (15-25°C) before use.

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## SPECIMEN COLLECTION AND PREPARATION

Osteocalcin in serum has been reported to be sensitive to proteolysis. It is recommended that blood be kept at 2-8°C immediately after collection and during processing. Serum should be processed and frozen at ≤ -20°C within 4 hours of collection. If collection and processing is performed at ambient temperature, serum must be processed and tested or frozen (≤ -20°C) within 2 hours of collection. Serum should be frozen at ≤ -80°C for storage longer than one month. Sample collection tubes containing EDTA must not be used, as the EDTA chelates Ca<sup>++</sup>, and the monoclonal anti-osteocalcin antibody is Ca<sup>++</sup> dependent.

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## REAGENT PREPARATION AND STORAGE

All reagents should be equilibrated to room temperature (20-25°C) prior to use.

1. Coated Strips  
Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table in Metra™ Osteocalcin 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 (20-25°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 10mL of 1X Wash Buffer. Allow the pellet to completely dissolve.
4. Osteocalcin Standards and Controls  
Within 1 hour of use, reconstitute Standards and Controls with 0.5mL of 1X Wash Buffer. Allow at least 15 minutes for the pellet to completely dissolve. Reconstituted Standards and Controls should not remain at room temperature for more than 2 hours. Freeze unused portion of Standards and Controls at ≤ -20°C. Do not freeze/thaw more than 4 times.
5. 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.

## OSTEOCALCIN ASSAY PROCEDURE

Metra™ Osteocalcin Test Kit part number 8002

Kit Contents	Qty/Vol	Part
Substrate Tablets	3 each	0012
Anti-Osteocalcin	15mL	4089
Standard A (OC 0ng/mL)	1 each	4168
Standard B (OC 2ng/mL)	1 each	4169
Standard C (OC 4ng/mL)	1 each	4170
Standard D (OC 8ng/mL)	1 each	4171
Standard E (OC 16ng/mL)	1 each	4172
Standard F (OC 32ng/mL)	1 each	4173
Control, Low	1 each	4174
Control, High	1 each	4175
Enzyme Conjugate (lyophilized)	3 each	4180
Coated Strips (12)	1 each	4670
Stop Solution	10mL	4702
10X Wash Buffer	55mL	4703
Substrate Buffer (3)	10mL	4705

### Materials Required BUT NOT Provided

Micropipettes to deliver 25-300µL  
Items suitable for liquid measurement of 10-300mL  
Container for wash buffer dilution  
Deionized or distilled water  
Plate reader  
4-parameter calibration curve fitting software

### Preparation of Reagents and Materials

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

# of Strips	4	6	8	12
# of Samples	8	16	24	40
(tested in duplicate)				
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 of each vial, and mix prior to use.

### Sample/Anti-Osteocalcin Incubation

1. Prepare required amount of 1X Wash Buffer (see table) by diluting 10X Wash Buffer 1:10 with deionized water. Store at room temperature (20-25°C). Use 1X Wash Buffer within 24 hours of preparation.
2. Within 1 hour of use, reconstitute Standards and Controls with 0.5mL of 1X Wash Buffer. Allow at least 15 minutes for the pellet to completely dissolve. Reconstituted Standards and Controls should not remain at room temperature for more than 2 hours. Freeze unused portion of Standards and Controls at  $\leq -20^{\circ}\text{C}$ . Do not freeze/thaw more than 4 times.
3. 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.
4. Place desired number of Coated Strips in the Stripwell Frame just prior to use. Label strips to prevent mix-up in case of accidental removal from Stripwell Frame.
5. Add 25µL of Standard, Control, or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
6. Add 125µL of Anti-Osteocalcin to each well and incubate for 2 hours ( $\pm 10$  minutes) at room temperature (20-25°C).
7. Prepare Enzyme Conjugate within 2 hours of use. Reconstitute each required vial of Enzyme Conjugate (see table) with 10mL of 1X Wash Buffer. Allow the pellet to completely dissolve.

## OSTEOCALCIN ASSAY PROCEDURE (CONT.)

### Enzyme Conjugate Incubation

1. Manually invert/empty strips. Add at least 300µL 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.
2. Add 150µL of the reconstituted Enzyme Conjugate to each well.
3. Incubate for 60 minutes ( $\pm 5$  minutes) at room temperature (20-25°C).
4. Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of Substrate Buffer (see table). Allow 30-60 minutes for the tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

### Substrate Incubation

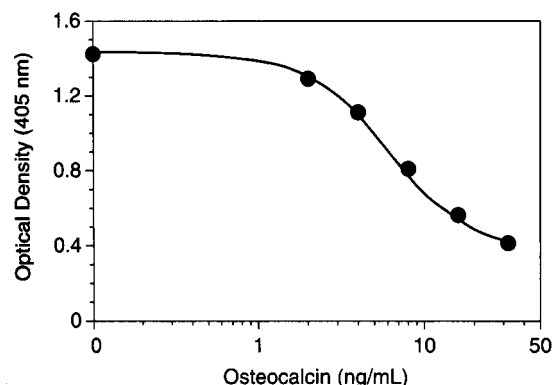
1. Manually invert/empty strips. Add at least 300µL of 1X Wash Buffer to each well and manually invert/empty strips. Repeat two more times for a total of three washes. Vigorously blot the strips dry on paper towels after the last wash.
2. Add 150µL of Working Substrate Solution to each well.
3. Incubate for 35-40 minutes at room temperature (20-25°C).

### Stop/Read

1. Add 50µL 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. Strips should be read within **15 minutes** of Stop Solution addition.
3. Quantitation software with a 4-parameter calibration curve fitting equation **must** be used to analyze Metra™ Osteocalcin assay results.

### Representative Standard Curve

Standard Osteocalcin levels: 0, 2, 4, 8, 16, 32 ng/mL



## INTERPRETATION OF RESULTS

Sample results are expressed as ng/mL and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).

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## LIMITATIONS

The Metra Osteocalcin Enzyme Immunoassay has been validated for manual washing procedures.

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## METRA™ OSTEOCALCIN EXPECTED VALUES

Preliminary Metra™ Osteocalcin reference ranges have been established for normal premenopausal females (n=79) and for normal males (n=61) over 25 years of age. For the purposes of establishing preliminary reference ranges, normal 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

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

Females: 3.7 - 10.0 ng/mL

Males: 3.4 - 9.1 ng/mL

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## PERFORMANCE CHARACTERISTICS

### Antibody Specifications

The monoclonal anti-osteocalcin antibody was raised against bovine osteocalcin which exhibits significant homology with human osteocalcin. This antibody is believed to be conformationally dependent and should thus recognize only intact (*de novo*) osteocalcin and not fragments from resorbed bone tissue.

	% Reactivity
Human intact osteocalcin	100
Bovine intact osteocalcin	100
Reduced, alkylated osteocalcin	ND
C-terminal osteocalcin fragment	ND
N-terminal osteocalcin fragment	ND
ND, not detected	

### Sensitivity

The minimum detection limit of the Metra™ Osteocalcin Assay is 0.45ng/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.

Osteocalcin ng/mL	Within-run <sup>1</sup> CV%	Between-run <sup>2</sup> CV%
6.2	10.0	9.8
7.4	4.8	4.8
16.5	8.0	7.6

<sup>1</sup> n=22      <sup>2</sup> n=3 in 3 runs

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