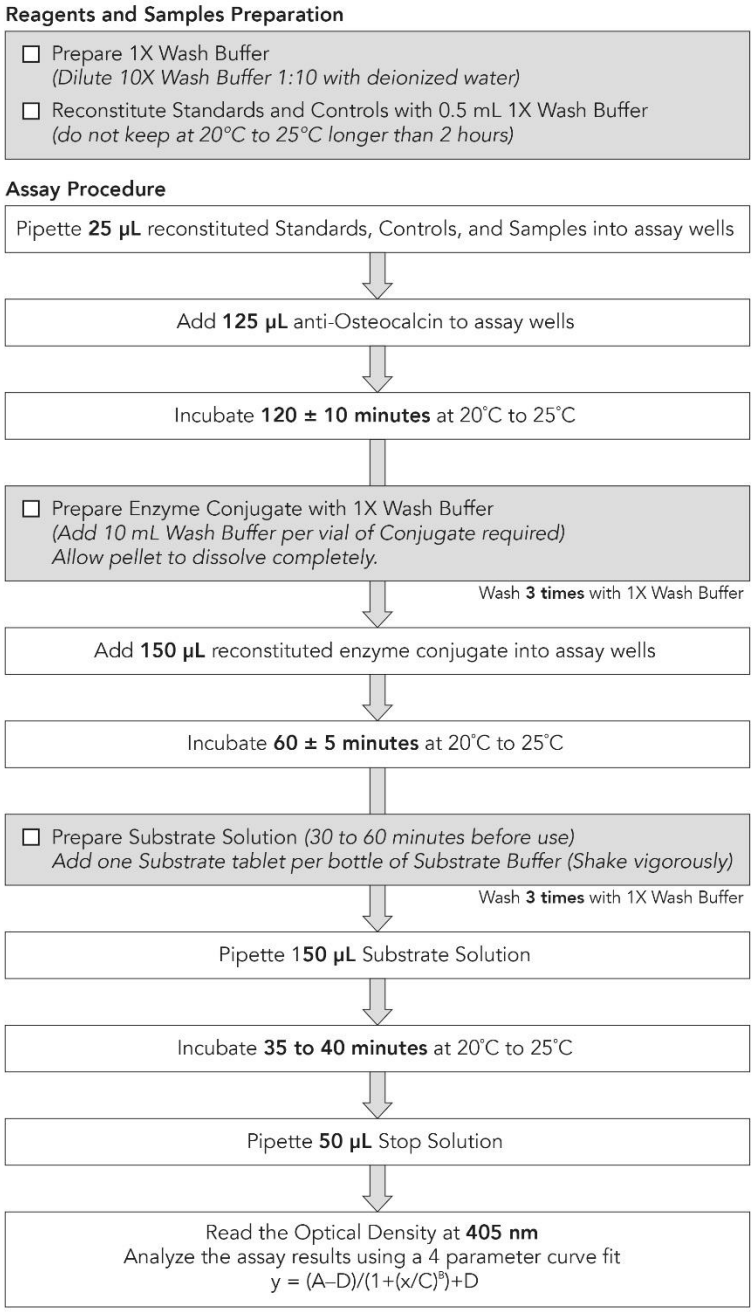
QUIDEL

MicroVue™ Bone Osteocalcin EIA

An enzyme immunoassay for the quantitation of intact osteocalcin in plasma or serum

SUMMARY



INTENDED USE

The MicroVue Osteocalcin immunoassay quantitatively measures intact (*de novo*) osteocalcin in serum or EDTA plasma. 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 that are thought to be involved in calcium ion and hydroxyapatite binding. It accounts for 10-20% of the non-collagenous protein in bone. While the *in vivo*function of osteocalcin is unknown, its affinity for bone mineral constituents implies a role in bone formation.

Osteocalcin makes up between 10 and 20% of non-collagenous protein in bone. The MicroVue Osteocalcin immunoassay quantitatively measures intact *(de novo)* osteocalcin in serum. Intact osteocalcin may be useful as a biochemical indicator of bone turnover. While the *in vivo* function of osteocalcin is unknown, its affinity for bone mineral constituents implies a role in bone formation. Elevated osteocalcin levels have been demonstrated in different diseases, including osteomalacia, Paget's disease of the bone, hyperthyroidism, primary hyperparathyroidism, and renal osteodystrophy. Osteocalcin levels can also be elevated in postmenopausal osteoporosis due to increased or decreased bone turnover. Depressed levels of osteocalcin have been reported in hypoparathyroidism and during long-corticosteroid therapy.

PRINCIPLE OF THE PROCEDURE

The MicroVue 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 plasma or serum.

REAGENTS AND MATERIALS PROVIDED

96 Assays for Osteocalcin
MicroVue Osteocalcin EIA kit contains the following:

A	**Osteocalcin Standards:**	**Parts 4168 – 4173**	**1 each**
B	**Standards A-F, lyophilized, concentration see Certificate of Analysis**		
C	Lyophilized osteocalcin, purified from human bone, containing buffer salts and stabilizers		
D			
E			
F			
L	**Low/High Controls**	**Parts 4174, 4175**	**1 each**
H	Lyophilized osteocalcin, purified from human bone, containing buffer salts and stabilizers		
❶	**Coated Strips**	**Part 4670**	**12 each**
	Osteocalcin purified from human bone adsorbed onto stripwells		
❷	**Stop Solution**	**Part 4702**	**15 mL**
	0.5N NaOH		
❸	**10X Wash Buffer**	**Part 4703**	**55 mL**
	Nonionic detergent in a buffered solution containing sodium azide (0.05%) as a preservative		

❹ **Anti-Osteocalcin** **Part 4089** **15 mL**
Purified murine monoclonal anti-osteocalcin antibody in a buffered solution containing nonionic detergent, stabilizers, 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 4180** **3 each**
Lyophilized goat anti-mouse IgG antibody conjugated to alkaline phosphatase containing buffer salts and stabilizers

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettes to deliver 25-300 µL and 500 µL
- Items suitable for liquid measurement of 10-300 mL
- Container for wash buffer dilution
- Wash bottle
- 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 in the U.S. 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 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 pipets or repeat pipettors is recommended to ensure the timely delivery of reagents.
- For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
- 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.
- Perform the Sample/Anti-Osteocalcin Incubation 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.
- Do not allow microassay wells to dry once the assay has begun.
- When removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.

- Adequate wash buffer volume is critical; dispense at least 300 µL per well in the wash steps. Perform this assay with any validated washing method. For best results, do not use a multichannel pipette to wash the plate.
- 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 the 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.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

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

SPECIMEN COLLECTION AND PREPARATION

Osteocalcin in serum or EDTA plasma has been reported to be sensitive to proteolysis. It is recommended that blood be kept at 2°C to 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 ≤ –70°C for storage longer than one month.

Thaw frozen (≤ –70°C) specimens rapidly in a 37°C water bath until just thawed. Transfer thawed specimens immediately to ice (for no longer than four hours). **Do not leave specimens at 37°C.** Do not thaw specimens at room temperature or 4°C. Frozen specimens should be tested as soon as possible after thawing. Repeated freezing and thawing is not recommended. If samples are to be refrozen for further analysis, we suggest freezing multiple aliquots of the specimen to prevent repeated freeze/thaw cycles.

REAGENT PREPARATION

All reagents should be equilibrated to 20°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 10 mL of 1X Wash Buffer. Allow the pellet to completely dissolve.

Osteocalcin Standards and Controls

Within 1 hour of use, reconstitute Standards and Controls with 0.5 mL of 1X Wash Buffer. Allow at least 15 minutes for the pellet to completely dissolve. Reconstituted Standards and Controls should not remain at 20°C to 25°C for more than 2 hours. Freeze unused portion of Standards and Controls at ≤ –20°C. Do not freeze/thaw more than 4 times.

Working Substrate Solution

Prepare Working Substrate Solution within 1 hour of use. Put one Substrate Tablet into each required bottle of 20°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 and WARNINGS AND PRECAUTIONS 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 (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/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 18°C to 28°C. Use 1X Wash Buffer within 21 days of preparation.
2. Within 1 hour of use, reconstitute Standards and Controls with 0.5 mL of 1X Wash Buffer. Allow at least 15 minutes for the pellet to completely dissolve. Reconstituted Standards and Controls should not remain at 20°C to 25°C for more than 2 hours. Freeze unused portion of Standards and Controls at ≤ –20°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 25 µL of Standard, Control, or sample to each well of the Coated Strips. This step should be completed within 30 minutes.
6. Add 125 µL of Anti-Osteocalcin to each well and incubate for 2 hours (± 10 minutes) at 20°C to 25°C.
7. While incubating, prepare Enzyme Conjugate. Reconstitute each required vial of Enzyme Conjugate (see table) with 10 mL of 1X Wash Buffer. Allow the pellet to completely dissolve. Use within 2 hours.

Washing Step (1)

8. 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

9. Add 150 µL of the reconstituted Enzyme Conjugate to each well.

10. Incubate for 60 minutes (± 5 minutes) at 20°C to 25°C.
11. While incubating, prepare Working Substrate Solution. 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. Use within one hour.

Washing Step (2)

12. 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

13. Add 150 µL of Working Substrate Solution to each well.
14. Incubate for 35 to 40 minutes at 20°C to 25°C.
    NOTE: If temperature cannot be maintained between 20°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 A wells; stop the reaction when the optical density reaches 1.5; then read the strips.

Stop/Read

15. Add 50 µL of Stop Solution to each well to stop the reaction.
16. 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 the strips within **15 minutes** of Stop Solution addition.
17. Use quantitation software with a 4-parameter calibration curve fitting equation to analyze MicroVue Osteocalcin assay results.

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

18. Determine concentration of samples and Controls from the standard curve. Dilute samples greater than 32 ng/mL in 1X Wash Buffer and retest. Include the dilution factor in the calculation. 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.

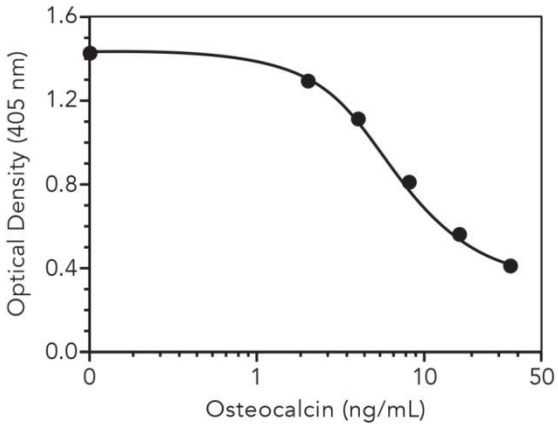
Quality control ranges are provided. The control values are intended to verify the validity of the curve and sample results. Each laboratory should establish its own parameters for acceptable assay limits. If the control values are NOT within your laboratory's acceptance limits, the assay results should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

If the optical density of the MicroVue Osteocalcin Standard A 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 ng/mL and **do not** need to be corrected for dilution (unless sample was diluted prior to testing).

Representative Standard Curve



EXAMPLE VALUES

In our testing of 140 adults over 25 years of age, values obtained from the MicroVue Osteocalcin kit ranged from 3.7 ng/mL to 10.0 ng/mL.

Values may be influenced by such factors as low estrogen production, low calcium intake, or low physical activity. Estrogen deficiency in post-menopausal women can result in elevated bone turnover. Each laboratory should establish its own normal reference range.

PERFORMANCE CHARACTERISTICS

Antibody Specificity

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

Limits of Detection

The minimum analytical detection limit of the MicroVue Osteocalcin Assay is 0.45 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.

Osteocalcin (ng/mL)	Within-run[1] C.V. (%)	Between-run[2] C.V. (%)
6.2	10.0	9.8
7.4	4.8	4.8
16.5	8.0	7.6

[1]n = 22 replicates [2]n = 3 in 3 runs

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**.

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REF 8002 – MicroVue Osteocalcin EIA Kit

IVD

CE

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

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GLOSSARY

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	IVD For *In Vitro* diagnostic use
 Contains sufficient for 96 determinations	CONT Contents/Contains
CONTROL Control	