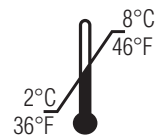


MICROVUE™ Helical Peptide EIA Kit

Bone Health

An enzyme immunoassay for the
quantitation of Helical Peptide 620-633
from the α 1 chain of Type I Collagen
in Urine

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



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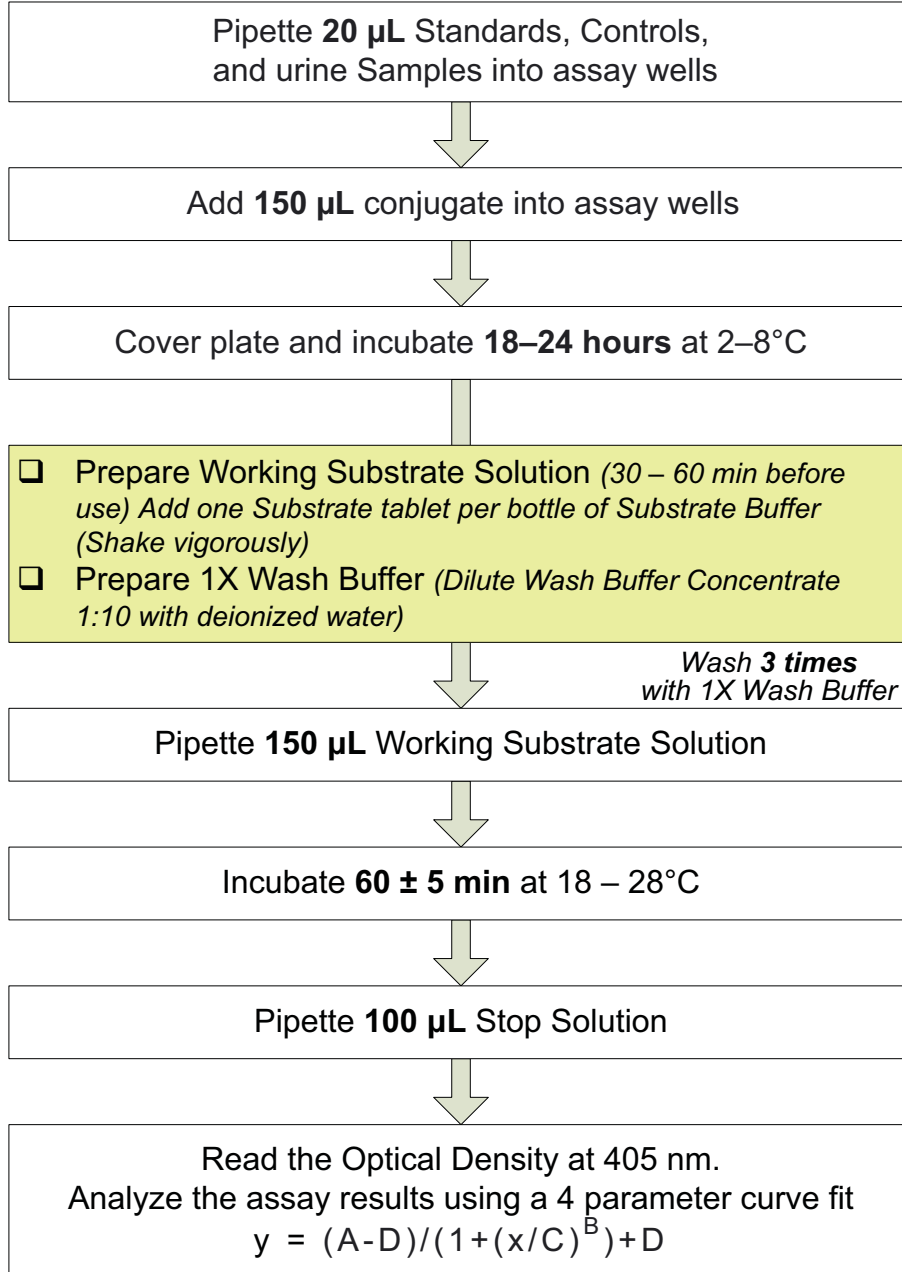
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MicroVue™ Helical Peptide EIA Summary

Reagents and Samples Preparation

- ❑ Prepare Enzyme Conjugate with Assay Buffer Solution, store at 2-8°C. (Add 10 mL Assay Buffer per each required vial of Enzyme Conjugate. Prepare within 2 hours of use.)

Assay Procedure



SUMMARY AND EXPLANATION

MicroVue Helical Peptide is a urinary assay that provides a quantitative measure of the excretion of a peptide consisting of residues 620–633 in the helical region of the type I collagen α 1 chain.

Bone is constantly undergoing a metabolic process called remodeling, or “turnover.”^{1,2} This includes a degradation process, bone resorption, mediated by the action of osteoclasts, and a building process, bone formation, mediated by the action of osteoblasts. Remodeling is required for the maintenance and overall health of bone. The measurement of specific degradation products of bone matrix provide analytical data of the rate of bone metabolism.^{1,2}

The organic matrix of bone contains approximately 90% of type I collagen, a helical, heterotrimeric molecule comprising two α 1 chains and one α 2 chain.² During bone resorption, the collagen molecule is degraded, releasing into the circulation peptides of various molecular weights that are further degraded and/or released into urine.^{1,2} A peptide consisting of residues 620–633 derived from the helical region of the α 1 chain was isolated from the urine of a patient with Paget’s disease.³ Using synthetic peptide α 1(I) 620–633 as an immunogen for production of monoclonal antibodies, a competitive immunoassay has been developed to measure this helical peptide in human urine for bone metabolism studies.^{3,4} Since helical peptide is present and excreted in all mammalian species evaluated, including rats, rabbits, guinea pigs, cats, dogs, pigs, sheep, cows, horses, and non-human primates, research applicability of the Helical Peptide immunoassay extends to animal model studies.

PRINCIPLE OF THE PROCEDURE

The MicroVue Helical Peptide assay is a competitive enzyme immunoassay in a microassay stripwell format utilizing a monoclonal anti-helical peptide antibody coated on the strip to capture helical peptide. Helical peptide in the sample competes with conjugated helical peptide-alkaline phosphatase for the antibody, and the reaction is detected with a pNPP substrate. Helical peptide results are corrected for urinary concentration by creatinine.

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. The Stop Solution (0.5N NaOH) is considered corrosive and can cause irritation. Do not ingest. Avoid contact with skin, eyes or clothing. If contact is made, immediately wash affected area with water. If ingested, call a physician.
8. 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.
9. The substrate buffer contains diethanolamine and may cause irritation to the eyes and/or skin with prolonged contact. Wear suitable protective clothing, gloves, and eye/face protection. Contacted areas should be immediately washed with soap and water.
10. Use of multichannel pipettes or repeat pipettors is recommended to ensure the timely delivery of reagents.
11. For accurate measurement of samples, add samples and standards precisely. Pipet carefully using only calibrated equipment.
12. Test each sample in duplicate.
13. Do not use a microassay well for more than one test.
14. Using incubation times and temperatures other than those indicated in the *ASSAY PROCEDURE* section may give erroneous results.
15. Do not allow microassay wells to dry once the assay has begun.

16. When adding or removing liquid from the microassay wells, do not scrape or touch the bottom of the wells.
17. Use a wash bottle or automated filling device to wash the plate. Do not use a multichannel pipette to wash the plate.
18. Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.

REAGENT PREPARATION

All reagents should be equilibrated to 18–28°C prior to use.

Wash Buffer

Prepare required amount of 1X Wash Buffer (see table in *ASSAY PROCEDURE* section) by diluting 10X Wash Buffer 1:10 with deionized water. Store at 18–28°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 Assay Buffer. Store reconstituted Enzyme Conjugate at 18–28°C until use.

Working Substrate Solution

The Substrate Buffer must be brought to 18–28°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 18–28°C Substrate Buffer (see table). Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

STORAGE

Store the kit at 2–8°C.

Store unused reagents at 2–8°C.

Store 1X Wash Buffer (10X diluted) at 18–28°C.

SPECIMEN COLLECTION AND PREPARATION

Collect preservative-free urine. It is recommended collections be made prior to 10:00 am to obviate any potential influence of diurnal variation. Keep the urine samples refrigerated (2–8°C) for storage of up to 7 days, or freeze the samples at less than -20°C for longer storage. Do not subject samples to more than 4 freeze/thaw cycles.

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.

Sample/Enzyme Conjugate Incubation

1. Allow pouch of Coated Strips to equilibrate to 18–28°C before opening. Remove Stripwell Frame and the required number of Coated Strips from the pouch (see table above). Ensure that the pouch containing any unused strips is completely resealed and contains desiccant.
2. Add 20 µL Standards, Controls, or urine samples to each well of the Coated Strips.
3. Prepare Enzyme conjugate within 2 hours of use. Reconstitute each required vial (see table) with 10 mL of Assay Buffer. Store reconstituted Enzyme Conjugate at 18–28°C until use.
4. Add 150 µL of 18–28°C reconstituted Enzyme Conjugate to each well. Cover strips with Tape Cover provided. Incubate 18–24 hours at 2–8°C.
5. Prepare Working Substrate Solution within 1 hour of use. Add 1 tablet to each required bottle (see table above) of 18–28°C Substrate Buffer. Allow 30–60 minutes for tablet(s) to dissolve. Vigorously shake bottle(s) to completely mix.

Substrate Incubation

6. Prepare required amount of 1X Wash Buffer (see table above) by diluting 10X Wash Buffer 1:10 with deionized water. Manually invert/empty strips. Add at least 275 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 bottoms of strips with a lint-free paper towel to ensure that the bottoms of the strips are clean.

7. Add 150 µL of Working Substrate Solution to each well. Incubate for 60 ± 5 minutes at 18–28°C. **Note:** If room temperature cannot be maintained between 18–28°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 strip(s).

Stop/Read

8. 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.
9. Read the absorbance 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 30 minutes of Stop Solution addition.
10. Quantitation software with a 4-parameter calibration curve fitting equation must be used to analyze the Helical Peptide assay results:

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

11. Plot a standard curve using the A_{405} value for each Helical Peptide Standard on the y-axis and the assigned Helical Peptide concentration (µg/L) for each Standard on the x-axis. Determine the concentration of urine samples and controls from the standard curve. Kit Control values should be within the range specified in the Certificate of Analysis supplied with the kit. Standard concentrations are assigned for each lot. Read label on each Standard vial or Certificate of Analysis carefully for specific concentrations. Dilute Urine samples greater than the concentration of Standard F in Assay Buffer and retest. Include the dilution factor in the final calculation.

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 Helical Peptide Standard A is less than 0.8, the results should be considered questionable, and the samples should be repeated.

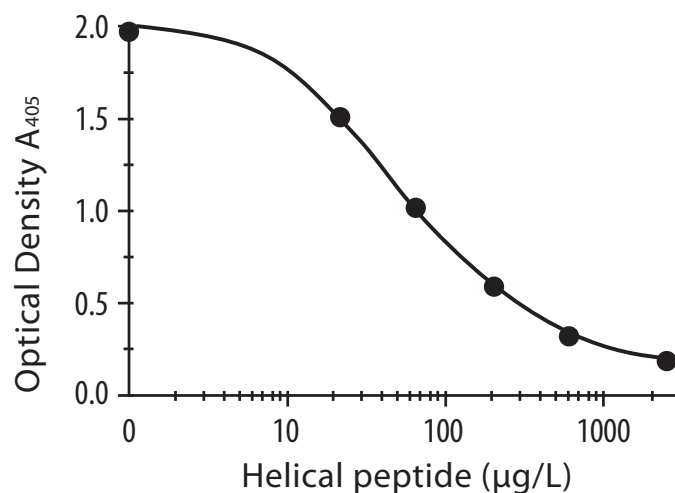
INTERPRETATION OF RESULTS

Results obtained from the MicroVue Helical Peptide assay must be corrected for variations in urine concentration by dividing the Helical Peptide value ($\mu\text{g/L}$) by the creatinine value (mmol/L) of each sample ($\text{creatinine mg/dL} \times 0.088 = \text{mmol/L}$). The final Helical Peptide results will be expressed as $\mu\text{g/mmol creatinine}$.

Representative Standard Curve

Standard Helical Peptide levels:

0, 20.2, 61.3, 203.3, 624.0, 2514 $\mu\text{g/L}$



EXAMPLE VALUES

In our testing of 100 adults aged 25–34 years, values obtained from the MicroVue Helical Peptide kit had a mean value of 50.7 $\mu\text{g/mmol Creatinine}$.

PERFORMANCE OF THE TEST

Specificity of the antibody

The anti-helical peptide antibody does not recognize intact forms of collagen types I, II, III, IV, or mixtures of amino acids at physiological levels. Homologous peptides from collagen types I, II, and III were synthesized to determine cross-reactivity with the anti-helical peptide antibody. The antibody exhibited ~90% cross-reactivity with $\alpha 1$ (III) homologous peptide. The antibody did not react with $\alpha 1$ (II), or $\alpha 2$ (I) homologous peptides.

Reactivity to Helical Peptide in Animal Urine

The helical peptide antibody demonstrates reactivity to helical peptide from the following animal species: rat, rabbit, guinea pig, cat, dog, pig, goat, sheep, cow, horse, rhesus and cynomolgus monkeys, baboon, and chimpanzee. The procedure for analyzing animal urines is the same as for human samples, except a minimum of 1:5 pre-dilution of animal samples with Assay Buffer is suggested.

Limits of Detection

The minimum detection limit of the MicroVue Helical Peptide Assay is 8 $\mu\text{g/L}$ as determined by the upper 3 SD limit in a zero standard study.

Precision

Within run and total precision was assessed according to a modified NCCLS (EP5-A) protocol. Four urine samples throughout the assay range were assayed. Duplicates were assayed by 3 operators in 20 runs using 2 lots of reagents.

Helical Peptide ($\mu\text{g/L}$)	Within-run CV (%)	Total CV (%)
22.4	8.1	16.9
79.5	6.5	8.6
235.7	4.5	8.6
855.2	4.0	7.3

Recovery - Dilution

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

	Average Recovery	Absolute Range
Human	100%	82 – 112%
Animal	99%	82 – 113%

Recovery - Spike Recovery

Spike recovery was determined by adding known quantities of helical peptide to urine samples of all listed species.

	Average Recovery	Absolute Range
Human	95%	88 –103%
Animal	101%	92 –121%

Interfering Substances

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

Substance	Concentration
pH	4 to 9
Glucose	2 g/dL
Bilirubin	0.25 mg/dL
Creatinine	500 mg/dL
Albumin	500 mg/dL
Hemoglobin	200 mg/dL
Sodium chloride	6 g/dL
Acetone	1 g/dL
Sodium azide	0.1% wt/vol
Boric acid	1% wt/vol
Sodium fluoride	0.13 mg/mL
Thimerosal	1% wt/vol
Urea	6 g/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.

REFERENCES

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3. Ju J, Leigh S, Byrne M, et al. A new marker for measuring bone resorption using an ELISA for a 14 amino acid peptide (residues 620-633) derived from the collagen α 1(I) chain. [Abstract]. *J.Bone Miner.Res.* 1997;12 (Suppl. 1): S178.
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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

REF 8022 – MicroVue™ Helical Peptide Enzyme Immunoassay Kit