TechNotes

HyTest

Clinical and Research Area

Miscellaneous

Osteocalcin

Osteocalcin (OC), which is also known as bone Gla protein (BGP), is a vitamin K-dependent bone matrix peptide of 49 amino acid residues. The concentration of OC in serum is considered to be an index of bone formation and numerous immunoassays for the measurement of OC have been developed in order to evaluate its potential role in reflecting metabolic disorders to bone. (1)

Although most of the synthesized human OC (hOC) is bound to bone hydroxyapatite, a small part of it leaks into the bloodstream. hOC concentrations in the circulation have been used in clinical investigations as a marker of bone formation. However, the discordant results obtained with different hOC assays have hindered the widespread usage of hOC in clinical applications. (1)

The protein contains three g-carboxyglutamic acids (Gla) that interact with Ca^{2}-ions on the surface of the hydroxyapatite, which is the major mineral component of bone. Although OC is predominantly associated with bone, a fraction of the newly synthesized protein is released into circulation where the status of the bone metabolism can be measured (3). The concentration of hOC increases in a variety of conditions that are characterized by increased bone turnover, such as osteoporosis, puberty, primary and secondary hyperparathyroidism, hyperthyroidism, and Paget's disease. Its concentration decreases correspondingly in hypothyroidism, hypoparathyroidism, as well as in patients receiving glucocorticoid treatment. (2)

The structure of osteocalcin is also well conserved in many vertebrate species. Therefore, it is difficult to raise antibodies against it in animals even if chemically coupled to various carrier molecules. On account of this it might be reasonable to produce recombinant osteocalcin as a fusion protein and subsequently use it as an immunogen for the development of new osteocalcin assays based on monoclonal antibodies (MAbs). In addition, the utilization of recombinant osteocalcin for the standardization of osteocalcin assays is also an advantage. This is because the purification of hOC from bone is a laborious and time-consuming procedure. (2)

1. Käkönen, S.M., Heterogenity of circulating forms of osteocalcin:Development of specific immunoassays for their determination. Dissertation 1999

2. Käkönen, S.M., et al., Development and evaluation of three immunofluorometric assays that measure different forms of osteocalcin in serum. Clin Chem. 2000 Mar;46(3):332-7.

3. Hellman, J., et al., Epitope mapping of nine monoclonal antibodies against osteocalcin: combinations into two-site assays affect both assay specificity and sample stability. Protein Expr Purif. 1996 Sep;8(2):137-44.

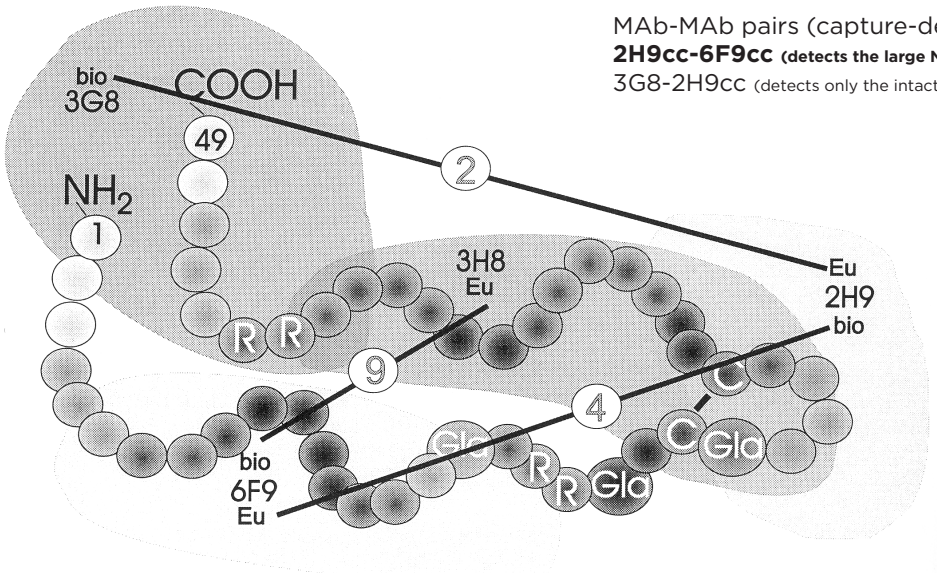
Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Osteocalcin, human	4OC8	3G7	IgG2b	EIA
		1C4	IgG1	EIA
		1C7	IgG1	EIA
		3G8	IgG1	EIA
		2H9cc	IgG2a	*In vitro,* EIA
		6F9cc	igG1	*In vitro,* EIA
		8H12	igG1	EIA

Monoclonal antibodies specific to osteocalcin

Two MAb-MAb combinations were selected on the basis of a previous study (2). Pair 3G8-2H9cc is specific to the intact hOC, whereas pair **2H9cc-6F9cc** also detects the large NH2- terminal fragment. (1) The precision profiles were obtained from 12 replicates of each calibrator of each assay (Fig. 1). The detection limits, which are defined as the concentration corresponding to the mean value of 12 determinations of the zero calibrator + 2 SD, were 0.01 and 0.008 nmol/L for IFMAs 3G8-2H9cc and 2H9cc-6F9cc respectively. The within-assay and between-assay variations were calculated with three serum samples (mean concentrations, 0.81, 3.30, and 5.28 nmol/L respectively, as measured with pair 3G8-2H9cc). The within-assay CVs were 5% (n = 12), while the between-assay CVs (n = 12) were 8% for each of the assays. (1)



MAb-MAb pairs (capture-detection):
2H9cc-6F9cc **(detects the large NH2- terminal fragment and intact hOC)**
3G8-2H9cc (detects only the intact hOC)

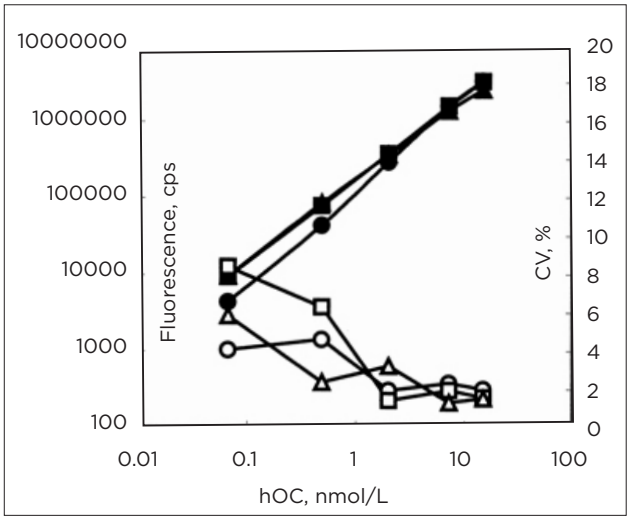


Figure 1. Dose-response curves of pairs 3G8-2H9 (■) and 2H9-6F9 (●) given as fluorescence counts vs concentrations of the hOC calibrator over the range 0.017-13.5 nmol/L and the corresponding within-assay CVs (open symbols) calculated from 12 replicates.

MAb	antigen	Ig-class	Eu-hOC	Eu-hOC 1-19	Eu-hOC 7-19	Eu-hOC 15-31	Eu-hOC 20-43	Eu-bOC
3G7	bOC	IgG2b	+	-	-	+	+	+
1C4	bOC	IgG1	+	-	-	+	+	+
1C7	bOC	IgG1	+	-	-	+	+	+
3G8	bOC	IgG1	(+)*	-	-	-	-	(+)*
2H9	rGST-hOC	IgG2a	+	-	-	+	+	+
6F9	rGST-hOC	IgG1	+	+	+	+	-	-
8H12	rGST-hOC	IgG1	+	+	+	+	-	+

Table 1. Summary of the human osteocalcin MAbs. The ability of the MAbs to recognize full length human OC, tryptic 1-19, 20-43, synthetic 7-19, 15-31 and bovine OC (bOC) was tested with Eu-labeled antigens. *MAb 3G8 recognizes unlabelled hOC and bOC when tested with two-site combinations.

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced in vivo. MAbs produced in vitro are expected to have similar performance.

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