Assay Solution
A Solution Provider

Bone Biomarker Overview

•Osteoporosis is a silent disease without obvious symptom and evidence until occurrence of fracture. Early diagnosis of osteoporosis is the key issue for efficient treatment and for identification of osteoporotic patient with high risk of fracture. At present, diagnosis of osteoporosis and assessment of fracture risk are based on the quantitative analysis of bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA). However, the gold standard method of BMD assessment of bone mass by DXA only partially provides the information about bone strength. The osteoporosis is characterized by bone fragility. Bone fragility is evaluated by its micro-architectural quality which is identified by all bone features such as microarchitecture, micro-damage and remodeling rates with the influence of bone's ability for resistant fracture.

•The biomarkers of bone turnover have been investigated in the past decade. As shown in the table below, the mechanism of bone remodeling is composed by bone resorption and bone formation. Bone biomarkers are produced from the bone remodeling process included bone formation biomarkers, bone resorption biomarkers and regulators of bone turnover. Detections of bone metabolism have been studied with the biomarkers of enzymes, proteins and by-products during the bone remodeling process. Various biomarkers are now available for specific and sensitive assessment of the rate for bone formation and bone resorption. For example, the bone formation biomarkers are total alkaline phosphatase (ALP), bone-specific alkaline phosphatase (BALP), osteocalcin (OC), procollagen type 1 N-terminal propeptide (P1NP) and procollagen type 1 C-terminal propeptide (P1CP). The bone resorption biomarkers are hydroxyproline (HYP), hydroxylysine (HYL), deoxypyridinoline (DPD), pyridinoline (PYD), bone sialoprotein (BSP), osteopontin (OP), tartrate-resistant acid phosphatase 5b (TRAP 5b), carboxy-terminal crosslinked telopeptide of type 1 collagen (CTX-1), amino-terminal crosslinked telopeptide of type 1collagen (NTX-1) and cathepsin K (CTSK). The regulators of bone turnover are receptor activator of NF-kB ligand (RANKL), osteoprotegerin (OPG), dickkopf-1 (DDK-1) and sclerostin. These biomarkers are useful to provide the early assessment of osteoporosis when the BMD measurement of DXA does not offer enough information to make the diagnosis. Therefore, the combination of BMD measurement by DXA and bone biomarker detections shows the great potential to improve the early assessment of people with the high risk of osteoporosis.

Bone formation biomarkers		
Human Total alkaline Phosphatase (ALP)	Alkaline Phosphatase (ALP) (liquid) assay	inquiry
Human Bone-specific alkaline Phosphatase (BALP)		

Human Pro-Collagen I alpha 1	Human Pro-Collagen I alpha 1 ELISA Kit	AYQ-E10376
PINP	Human PINP RIA kit	inquiry
	Human PINP Chemiluminescence Immunoassay kit	inquiry
	Rat/Mouse PINP ELISA kit	inquiry
Human PICP		
Human Osteocalcin (OC)	Human Osteocalcin ELISA Kit	AYQ-E11200
	Human N-Mid osteocalcin fragments ELISA kit	inquiry
	Osteocalcin Antibody	ASA-B1437
Bone Resorption biomarkers		
Hydroxyproline (HYP)		
Hydroxylysine (HYL)		
Deoxypyridinoline (DPD)	EIA kit	inquiry
Pyridinoline (PYD)		
Bone Sialoprotein (BSP)		
Osteopontin (OP)	Human Osteopontin (OPN) ELISA Kit	AYQ-E11161
	Osteopontin Antibody	ASA-B1438
	Osteopontin Antibody	ASA-B1439
	Osteopontin Antibody	ASA-B1440
Tartrate-resistant acid Phosphatase 5b (TRAP 5b)	Human Trap 5b ELISA kit	inquiry
	Mouse Trap 5b ELISA kit	inquiry

	Rat Trap 5b ELISA kit	inquiry
Carboxy-terminal Crosslinked Telopeptide of type 1 collagen (CTX-1),	Human CTX-1 ELISA kit	inquiry
	Mouse CTX-1 ELISA kit	inquiry
	Rat CTX-1 ELISA kit	inquiry
Amino-terminal Crosslinked Telopeptide of type 1 collagen (NTX-1)		
Cathepsin K (CTSK)		
Regulators of bone turnover		
Receptor activator of NF-κB ligand (RANK L)	Human TRANCE/RANK L/TNFSF11 ELISA Kit	AYQ-E10898
	sRANKL Human protein	AS-P05228
	sRANKL Human, His	AS-P05230
	sRANKL Human, GST	AS-P05229
	sRANKL Mouse	AS-P05231
Osteoprotegerin (OPG)	Human Osteoprotegerin/TNFRSF11B ELISA Kit	AYQ-E10925
	Osteoprotegerin Antibody	ASA-B1441
Dickkopf-1 (DKK-1)	Human Dkk-1 ELISA Kit	AYQ-E10902
	DKK1 Human	AS-P01388
	DKK1 Antibody	ASA-B0594
Sclerostin	Human SOST/Sclerostin ELISA Kit	AYQ-E10975
	SOST Human	AS-P05186
Others		

Intact PTH	Intact PTH ELISA Test kit	AYQ-RT-B02
25-OH Vitamin D (total)	25-OH Vitamin D (total) ELISA Test kit	AYQ-RT-B01
Calcium-sensing receptor (CaSR)	Human Anti-CaSR Autoantibody ELISA Kit	AYQ-EP-B01
Carbamylated Proteins antibody	Anti-CarP Antibody ELISA Kit	inquiry
Cyclic Cirtullinated Peptide antibody	Anti-Cyclic Cirtullinated Peptide (Anti-CCP) ELISA Kit	inquiry
Citrullinated Fibrinogen antibody	Anti-Citrullinated Fibrinogen(Anti-CFG) ELISA Kit	inquiry

•Alkaline phosphatase (ALP, ALKP, ALPase, Alk Phos) (EC 3.1.3.1) or basic phosphatase is a homodimeric protein enzyme of 86 kilodaltons. Each monomer contains five cysteine residues, two zinc atoms, and one magnesium atom crucial to its catalytic function, and it is optimally active at alkaline pH environments.ALP has the physiological role of dephosphorylating compounds. The enzyme is found across a multitude of organisms, prokaryotes and eukaryotes alike, with the same general function but in different structural forms suitable to the environment they function in. Alkaline phosphatase is found in the periplasmic space of E. coli bacteria. This enzyme is heat stable and has its maximum activity at high pH. In humans, it is found in many forms depending on its origin within the body – it plays an integral role in metabolism within the liver and development within the skeleton. Due to its widespread prevalence in these areas, its concentration in the bloodstream is used by diagnosticians as a biomarker in helping determine diagnoses such as hepatitis or osteomalacia.

•The level of alkaline phosphatase in the blood is checked through the ALP test, which is often part of routine blood tests. The levels of this enzyme in the blood depend on factors such as age, gender, blood type. Blood levels of alkaline phosphatase also increase two to four times during pregnancy. This is a result of additional alkaline phosphatase produced by the placenta. Additionally, abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder or bones. Kidney tumors, infections as well as malnutrition has also shown abnormal level of alkaline phosphatase in blood. Alkaline phosphatase levels in a cell can be measured through a process called "The scoring method". "The scoring method" is a technique used where a sample of the enzyme is extracted from the inside of blood cells and is analyzed and compared for varying enzyme activity. A blood smear is usually taken and undergoes differential centrifugation to isolate leukocytes and staining to categorize each leukocyte into specific “leukocyte alkaline phosphatase indices.” This marker is designed to distinguish leukocytes and determine different enzyme activity from each sample's extent of staining.

•Recent study has also shown that the activity of serum total ALP >129 U/L is used as an indicator for osteoporosis in men. Moreover, the decrease of total ALP has been demonstrated with the treatment with alendronate from 79.7 U/L to 64.8 U/L.

The results indicate that total ALP can be used as an indicator to reflect the efficiency of drug treatment for osteoporosis. However, small change and wide extensive range of total ALP may be led to wrong diagnosis of osteoporosis for postmenopausal women.

•Bone alkaline phosphatase (BAP) is the bone-specific isoform of alkaline phosphatase. A glycoprotein that is found on the surface of osteoblasts, BAP reflects the biosynthetic activity of these bone-forming cells. BAP has been shown to be a sensitive and reliable indicator of bone metabolism.

•Normal bone is constantly undergoing remodeling in which bone degradation or resorption is balanced by bone formation. This process is necessary for maintaining bone health. If the process becomes uncoupled and the rate of resorption exceeds the rate of formation, the resulting bone loss can lead to osteoporosis and, consequently, a higher susceptibility to fractures. Osteoporosis is a metabolic bone disease characterized by low bone mass and abnormal bone microarchitecture. It can result from a number of clinical conditions including states of high bone turnover, endocrine disorders (primary and secondary hyperparathyroidism and thyrotoxicosis), osteomalacia, renal failure, gastrointestinal diseases, long-term corticosteroid therapy, multiple myeloma, and cancer metastatic to the bones. Paget disease is another common metabolic bone disease caused by excessive rates of bone remodeling resulting in local lesions of abnormal bone matrix. These lesions can result in fractures or neurological involvement. Antiresorptive therapies are used to restore the normal bone structure.

Males

<2 years: 25-221 mcg/L

2-9 years: 27-148 mcg/L

10-13 years: 35-169 mcg/L

14-17 years: 13-111 mcg/L

Adults: < or =20 mcg/L

Females

<2 years: 28-187 mcg/L

2-9 years: 31-152 mcg/L

10-13 years: 29-177 mcg/L

14-17 years: 7-41 mcg/L

Adults

Premenopausal: < or =14 mcg/L

Postmenopausal: < or =22 mcg/L

•Osteocalcin, also known as bone gamma-carboxyglutamic acid-containing protein, is a small protein composed by 49 amino acids. OC is synthesized by mature osteoblasts, odontoblasts and hypertrophic chondrocytes. Moreover, OC is the most abundant non-collagenous protein in bone comprised about 2% of total protein in the human body. OC produced by osteoblasts plays an important role for metabolic regulation, bone mineralization and calcium ion homeostasis. OC has been demonstrated that the level of serum OC is highly correlated with the increase of BMD during treatment with bone formation drugs for osteoporosis. Serum OC has been considered a specific biomarker of osteoblast function for evaluation of bone formation rate in osteoporosis. In many studies, osteocalcin has been demonstrated as an important biomarker to investigate the efficiency for the drug on bone formation. For instance, with the RNAse-enriched-LF supplementation, the change of OC from baseline (7.2 ± 2.8%) has shown a promising and favorable effect in postmenopausal women. Furthermore, the mean levels of osteocalcin have revealed a significant difference between the postmenopausal osteoporotic (16.16 ± 4.5 ng/ml) and non-osteoporotic (11.26 ± 3.07 ng/ml) women. The uses of osteocalcin as a bone formation biomarker could provide the advantages such as tissue specificity, wide availability and low variation. The bone remodeling biomarker of serum OC may be useful for the assessment of osteoporosis and for the prediction of the fracture risk in elderly persons, especially in women.

•Type 1 collagen can be found in the organic bone matrix (> 90%), which is developed in bone from procollagen type 1. Procollagen type 1 is synthesized by fibroblasts and osteoblasts. Procollagen type 1 has N-terminal and C-terminal extensions, which are removed by specific proteases during conversion of procollagen to collagen. The procollagen type 1 included P1CP and P1NP are subsequently conjugated onto the bone matrix. The bone formation biomarker of P1NP is a specific indicator of type 1 collagen deposition. P1NP is released during the formation of type 1 collagen into the intracellular space and P1NP eventually exists in the blood stream. P1NP is usually released in the trimeric structure (derived from the trimeric collagen structure) and then is rapidly broken down to a monomeric form by thermal degradation effects. Antibodies of P1NP are used to detect the trimeric structure of P1NP by enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay. Recently, for the postmenopausal women with osteoporosis participating in the Parathyroid Hormone and Alendronate for Osteoporosis study, the mean value (54.1 μg/L) of total P1NP before initiating therapy is 74% higher than that in healthy premenopausal women with the age above 35. P1NP has been demonstrated to be a more sensitive bone biomarker to measure the bone formation rate in osteoporosis. The measurement of P1NP is being developed for clinical application.

•P1CP is a single protein with molecular weight of 115 kDa containing mannose-rich carbohydrate side chains inserted post-translationally. PICP is cleared by liver endothelial cells via the mannose receptor and therefore has a short serum half-life of 6–8 min. Recent study shows that the P1CP in serum is used as a biomarker of bone formation to evaluate the effect of nandrolone decanoate and female sex hormones. The mean initial PICP concentration in the women with a vertebral fracture (97 ± 23 ng/mL) is significantly lower than that in the women with a forearm fracture (116 ± 27 ng/mL). Currently, both of P1NP and P1CP are analyzed by specific immunoassays. However, for the analyses of procollagen type 1, the biomarker of P1NP is more extensively investigated in the literature than that of P1CP. Several studies have also demonstrated good correlations between serum P1CP levels and the rate of bone formation

•DPD is a molecule to mechanically stabilize collagen by crosslinking between individual collagen peptides . During the process of bone resorption, the crosslinked collagens are proteolytically broken down and then the DPD is released into the circulation

and excreted by urine. Most of DPD are found in the bone and dentin. Therefore, DPD is used as a specific biomarker for bone resorption. In previous work, the DPD has been pretreated with preanalytical hydrolysis and extraction before HPLC analysis because DPD is excreted in the urine in free (40%) and peptide-bound (60%) forms. To improve the accuracy, the peptide-bound form is transferred into free form for the HPLC measurement. The drawbacks for HPLC measurement of DPD are complicated procedure and variable recovery. Recently, the automated chemiluminescence immunoassay and enzyme immunoassay have been developed for the direct detection of urinary free DPD. The experimental results of chemiluminescence immunoassay and enzyme immunoassay methods have shown the correlation with HPLC measurement of urinary free DPD. The measurements of free DPD in urine by the immunoassay approaches have provided the possibility for the clinical application in the monitoring of patients with bone pathology and metabolic bone disease.

•Osteopontin (OPN) belongs to a family of secreted acidic proteins whose members have an abundance of negatively charged amino acids such as Asp and Glu. OPN also has a large number of consensus sequence sites for post-translational phosphorylation of Ser residues to form phosphoserine, providing additional negative charge. Contiguous stretches of high negative charge in OPN have been identified and named the polyAsp motif (poly-aspartic acid) and the ASARM motif (acidic serine- and aspartate-rich motif), with the latter sequence having multiple phosphorylation sites. This overall negative charge of OPN, along with its specific acidic motifs and the fact that OPN is an intrinsically disordered protein allowing for open and flexible structures, permit OPN to bind strongly to calcium atoms available at crystal surfaces in various biominerals. Such binding of OPN to various types of calcium-based biominerals – such as calcium-phosphate mineral in bones and teeth, calcium-carbonate mineral in inner ear otoconia and avian eggshells, and calcium-oxalate mineral in kidney stones– acts as a mineralization inhibitor to regulate crystal growth.

•OPN is a substrate protein for a number of enzymes whose actions may modulate the mineralization-inhibiting function of OPN. PHEX (phosphate-regulating gene with homologies to endopeptidases on the X chromosome) is one such enzyme, which extensively degrades OPN, and whose inactivating gene mutations (in X-linked hypophosphatemia, XLH) lead to altered processing of OPN such that inhibitory OPN cannot be degraded and accumulates in the bone (and tooth) extracellular matrix, likely contributing locally to the osteomalacia (soft hypomineralized bones) characteristic of XLH.

•Along with its role in the regulation of normal mineralization within the extracellular matrices of bones and teeth,OPN is also upregulated at sites of pathologic, ectopic calcification– such as for example, in urolithiasis and vascular calcification – presumably at least in part to inhibit debilitating mineralization in these soft tissues.

•Osteopontin has been implicated as an important factor in bone remodeling. Specifically, research suggests it plays a role in anchoring osteoclasts to the mineral matrix of bones. The organic part of bone is about 20% of the dry weight, and counts in, other than osteopontin, collagen type I, osteocalcin, osteonectin, bone sialo protein, and alkaline phosphatase. Collagen type I counts for 90% of the protein mass. The inorganic part of bone is the mineral hydroxyapatite, Ca10(PO4)6(OH)2. Loss of this mineral may lead to osteoporosis, as the bone is depleted for calcium if this is not supplied in the diet.

•OPN serves to initiate the process by which osteoclasts develop their ruffled borders to begin bone resorption. It is also found in urine, where it inhibits kidney stone formation.

•TRAP 5b is normally secreted by osteoclasts during bone resorption. Thus, TRAP 5b is used as a reference for osteoclast activity and numbers. In the circulation, the hydrolyzed TRAP 5b is metabolized in the liver and then excreted in the urine. TRAP 5b can be specifically detected in serum by immunoassays. Previous report shows that serum TRAP 5b has been used to identify limited or extensive bone metastasis in breast cancer patients. Furthermore, serum TRAP 5b has been applied to monitor the efficiency of alendronate treatment. The bone resorption biomarker of TRAP 5b is extensively studied and revealed good specificity and high sensitivity in comparison with other bone biomarkers.

•Telopeptides of type 1 collagen are extensively investigated and used bone resorption biomarkers included carboxy-terminal crosslinked (CTX-1) and amino-terminal crosslinked (NTX-1). CTX-1 and NTX-1 are both are released during collagen degradation. ELISA is used to measured CTX-1 with a monoclonal antibody against an octapeptide sequence (EKAHD-β-GGR) in the α-1 (I) chain of the β-isoform. Recent study has shown that CTX-1 is a specific and sensitive biomarker of bone resorption that can rapidly indicate the response to bisphosphonate therapy for postmenopausal osteoporosis. However, serum CTX-1 is influenced by food intake and blood withdrawal must take place in the fasting state because food intake substantially decreases the levels of CTX-1.

•During the process of bone remodeling, osteoblasts produce RANKL and OPG to regulate the differentiation and maturation of osteoclasts. Recently, dextromethorphan has been demonstrated the inhibition of RANKL-induced osteoclastogenesis and bone resorption by abrogating the activation of NF-κB signaling in vitro. The oral administration of dextromethorphan improves ovariectomy-induced osteoporosis in vivo. Serum levels of RANKL from humans have been observed for assessments of the states in metabolic bone diseases. Although the serum RANKL has been studied for fracture risk prediction and evaluation of the response from osteoporosis treatment, many works still need to be investigated for the clinical application of RANKL.

•OPG is generally considered to be a secreted soluble receptor and is produced by many different tissues and cell types including osteoblasts. The role of OPG is used as a decoy receptor for RANKL and inhibitor of osteoclastogenesis. Studies in mice have revealed that the OPG knockout mouse develops severe osteoporosis, whereas the overexpression of OPG in transgenic mouse models and OPG treatment of normal mice leads to osteopetrosis. OPG can be measured in serum, plasma EDTA, citrate and heparin samples. There are commercially available sandwich ELISA assays for analyzing OPG by using a monoclonal capture and polyclonal detection antibodies. However, the clinical use of serum OPG as a biomarker for evaluation of bone disease activity still needs additional demonstration.

•DKK-1 and sclerostin are the inhibitors of Wnt signaling and are applied as bone remodeling biomarkers. DKK-1 is produced by osteoblasts and is secreted into circulation. The serum levels of DDK-1 reflect the inhibition of bone formation. DKK-1 levels have decreased from 34.3 pmol/L at baseline to 29.7 pmol/L at the 24-month of the breast cancer patients with anastrozole

treatment. DKK-1 has shown the correlation with the BMD of the femoral neck and of the total hip. Further long-term studies are necessary to identify the clinical application of the regulator DKK-1 as a biomarker for assessment of osteoporosis.

•In the presence of sclerostin, the Wnt pathway is downregulated and consequently osteoblastic differentiation is inhibited. Sclerostin is produced by osteocytes. Sclerostin is secreted into circulation, and serum levels reflect inhibition of bone formation. In previous work, the concentrations of serum sclerostin have significantly increased from 29.5 pmol/L at baseline to 43.2 pmol/L after 24 months of treatment with anastrozole in breast cancer patients. However, the clinical trial is further needed for the use of sclerostin as a biomarker of bone turnover.