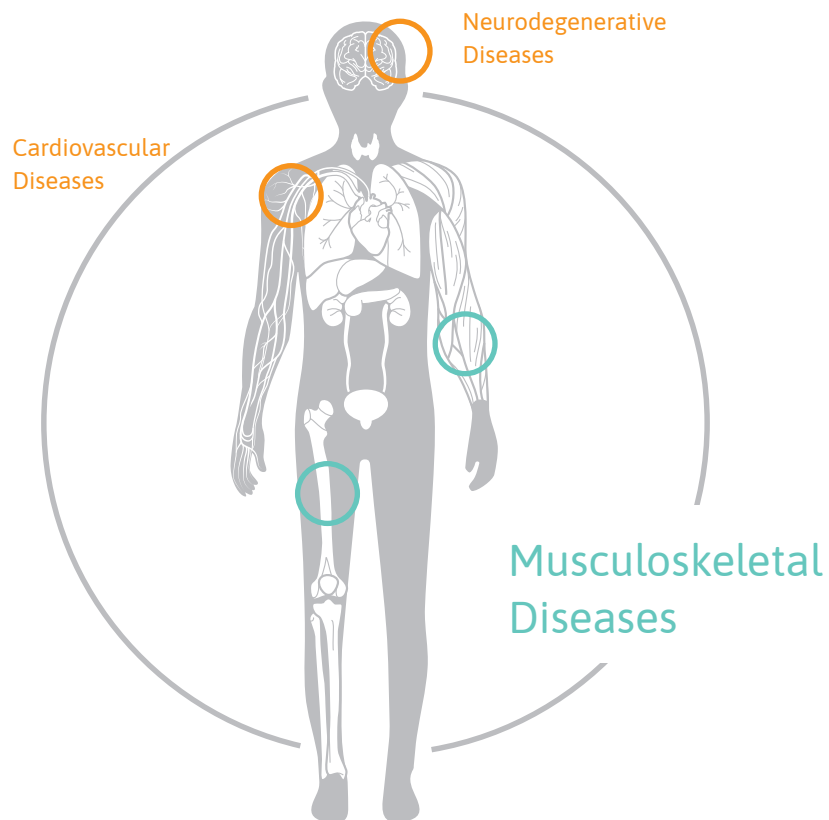


osteomiR™

microRNA Biomarkers of Bone Quality



a simple and standardized kit
qPCR analysis of validated
microRNA bone biomarkers



Circulating microRNAs are a novel class of blood-borne biomarkers. They are secreted from virtually any cell in the human body and distributed to other cells via the circulation. Local pathophysiologic processes in tissues can be detected using circulating microRNAs, and used for diagnosis and treatment monitoring of age-associated diseases.

The osteomiR™ kit enables the simple and standardized analysis of specific circulating microRNAs in human serum and plasma, which reflect bone quality.

osteomiRs – validated bone biomarkers

- Are associated to bone microstructure and histomorphometry
- Are significantly regulated in patients with osteoporotic fractures
- Are BMD-independent risk factors for osteoporotic fracture
- Regulate bone formation and resorption via multiple pathways
- Are novel biomarkers for bone disease, which can easily be detected in serum and plasma



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microRNA Biomarkers
of Bone Quality



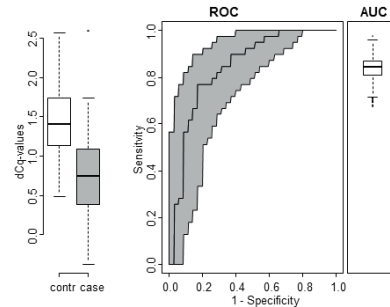
A novel molecular diagnostic test to detect high imminent fracture-risk in osteoporosis

19 individual bone-related biomarkers with distinct information content

A diagnostic algorithm, developed by TAMiRNA and SimplicityBio™, converts microRNA abundance into an individual fracture risk score

osteomiRs discriminate between fractured patients and controls

Selected model Input
10 microRNAs converted to 6 self-normalizing ratios



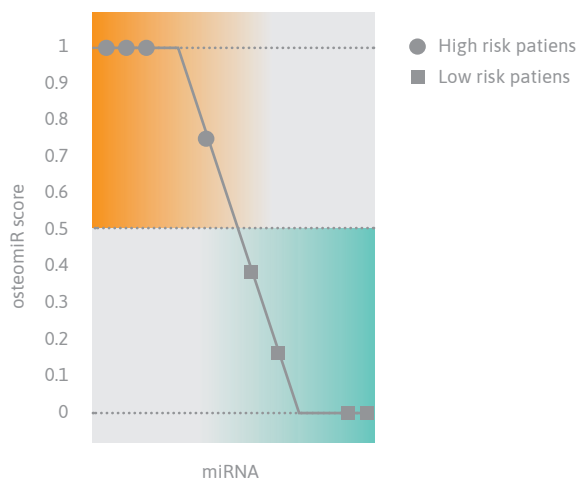
Model consisting of 4 rules

1. If Ratio 1 is low AND Ratio 2 is low, then high risk
2. If Ratio 3 is high, then low risk
3. If Ratio 4 is high AND Ratio 5 is low, then low risk
4. If Ratio 2 is high AND Ratio 6 is high, then high risk

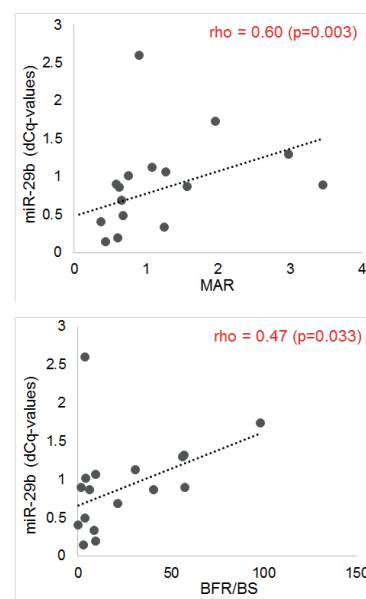
Box plots (left) show distribution of normalized miRNA-29b-3p serum levels in controls (n=36) and cases (n=39). ROC curves show ability to differentiate between cases and controls. Box plots (right) show AUC distribution based on 2000 bootstraps

Model Output

Fracture Risk Score



osteomiRs correlate with bone histomorphometric parameters

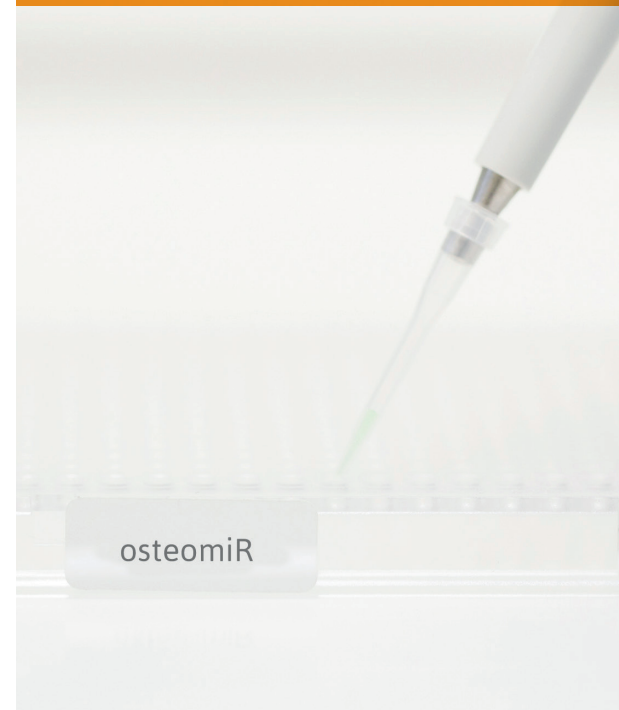
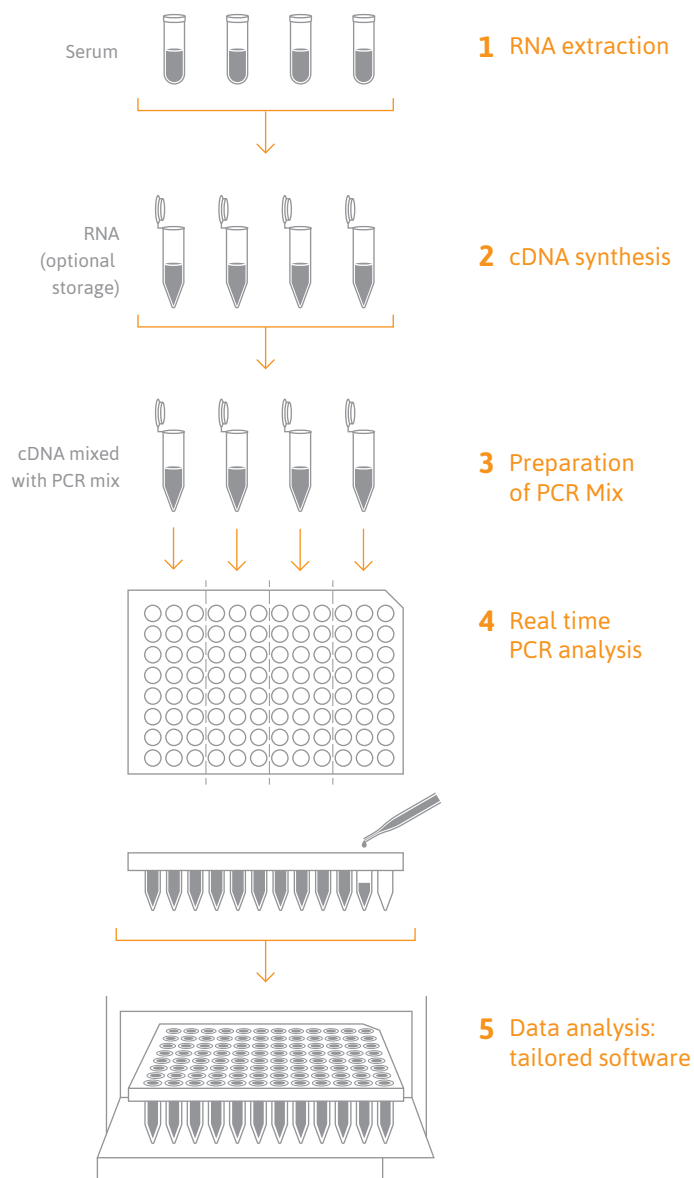


Relationship between miRNA levels and mineral apposition rate (MAR, top) bone surface/bone volume (BS/BV) and bone formation rate BFR/BS, below) based on 36 patients of postmenopausal and idiopathic osteoporosis

How does it work?



All-In-One kit:
the osteomiR™ kit includes all reagents for the following steps:



Assay format

- **Low sample volume:**
200 µL human serum/plasma
- **Fracture risk analysis utilizing the osteomiR™ signature:**
19 osteomiRs™ and 5 controls/sample
Customization is possible
- **Reduced hands-on time:**
primer coated 96 or 384 well plates
- **High throughput:**
analysis of up to 48 samples
(4 samples/plate, one kit includes 12 plates)
- **Fast and simple data analysis:**
osteomiR™ software included to obtain normalized data and a fracture-risk score

All osteomiRs™ have carefully been selected based on a series of clinical studies in the context of

- Bone turnover¹
- Microstructure and Histomorphometry²
- Osteoporosis & other bone diseases^{3,4,5}
- Bone Loss & Treatment Response^{1,6}
- Calcification^{4,7} and
- Therapeutic Activity^{7,8}

The osteomiR™ qPCR kit offers an easy solution for standardized analysis of 5 quality controls and 19 microRNA bone biomarkers in 200 µl serum.

Summary of evidence for microRNA bone biomarkers included in the osteomiR™ kit

miRNA ID	Bone Turnover	Microstructure and Histomorphometry	Osteoporosis & other bone diseases	Bone Loss & Treatment Response	Calcification	Therapeutic Activity	Mechanism of action
let-7b-5p	•	•	•				VEGF, HMGA2
miR-127-3p			•				S1PR3
miR-133b	•		•	•			Runx2/FOXC1
miR-141-3p			•		•		WNT
miR-143-3p			•				17β-estradiol, osterix
miR-144-5p	•						RANK
miR-152-3p			•				
miR-17-5p				•	•		Smad5, BMP2
miR-188-5p			•	•		•	PPARγ via HDAC9/RICTOR
miR-19b-3p	•		•	•			
miR-203a			•	•	•		Runx2, Dlx5, 17β-estradiol
miR-214-3p					•	•	ATF4
miR-29b-3p	•	•	•		•		HDAC4, TGFβ3, CTNNBIP1
miR-31-5p			•	•	•	•	WNT via FZD3
miR-320a			•		•		HOXA10
miR-335-5p	•	•	•	•	•		WNT via DKK1
miR-375			•		•	•	WNT via LRP5 and β-catenin
miR-550a-3p	•	•	•		•		
miR-582-5p			•				

Key publications

1. Anastasilakis AD et al. Changes of Circulating MicroRNAs in Response to Treatment With Teriparatide or Denosumab in Postmenopausal Osteoporosis. J Clin Endocrinol Metab. 2018 Mar 1;103(3):1206-1213.
2. Feichtinger X, et al. Bone-related Circulating MicroRNAs miR-29b-3p, miR-550a-3p, and miR-324-3p and their Association to Bone Microstructure and Histomorphometry. Sci. Rep. 2018 20;8(1):4867.
3. Kocijan R, Muschitz C, et al. Circulating microRNA signatures in patients with idiopathic and postmenopausal osteoporosis and fragility fractures. J Clin Endocrinol Metab. 2016 Aug 23.
4. Heilmeyer U, Hackl M, et al. Serum microRNAs are indicative of skeletal fractures in postmenopausal women with and without type-2 diabetes and

- influence osteogenic and adipogenic differentiation of adipose-tissue derived mesenchymal stem cells in vitro. J Bone Miner Res 2016 Jun 27.
5. Mäkitie RE, Hackl M, et al. Altered MicroRNA Profile in Osteoporosis Caused by Impaired WNT Signaling. JCEM 2018 ;Mar 1.
6. Kocijan et al., Time-dependent Analysis of microRNAs and Bone Microstructure under consideration of Anti-osteoporotic Treatment. Unpublished
7. Weilner S, et al. Secreted microvesicular miR-31 inhibits osteogenic differentiation of mesenchymal stem cells. Aging Cell 2016; 1-11.
8. Li D, et al, Osteoclast-derived exosomal miR 214-3p inhibits osteoblastic bone formation. Nat Commun, 2016, Mar 7;7:10872.

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