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

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

**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

**Model Output**
- Fracture Risk Score

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**19 individual bone-related biomarkers with distinct information content**

**osteomiRs**
- Discriminate between fractured patients and controls
- Correlate with bone histomorphometric parameters

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**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**

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**How does it work?**

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

1. **RNA extraction**
2. **cDNA synthesis**
3. **Preparation of PCR Mix**
4. **Real time PCR analysis**
5. **Data analysis: tailored software**

**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 turnover
- Microstructure and Histomorphometry
- Osteoporosis & other bone diseases
- Bone Loss & Treatment Response
- Calcification and
- Therapeutic Activity

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

<table>
<thead>
<tr>
<th>miRNA ID</th>
<th>Bone Turnover</th>
<th>Microstructure and Histomorphometry</th>
<th>Osteoporosis &amp; other bone diseases</th>
<th>Bone Loss &amp; Treatment Response</th>
<th>Calcification</th>
<th>Therapeutic Activity</th>
<th>Mechanism of action</th>
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<tbody>
<tr>
<td>let-7b-5p</td>
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### Key publications

6. Kocijan et al., Time-dependent Analysis of microRNAs and Bone Microstructure under consideration of Anti-osteoporotic Treatment. Unpublished
A novel molecular diagnostic test to detect high imminent fracture-risk in osteoporosis

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OsteomiRs discriminate between fractured patients and controls.

OsteomiRs correlate with bone histomorphometric parameters.

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.