a simple and standardized kit
qPCR analysis of circulating platelet-derived microRNAs
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 pathophysiological processes in tissues can be detected using circulating microRNAs, and used for diagnosis and treatment monitoring of age-associated diseases.

The thrombomiR™ kit enables simple and standardized analysis of microRNA biomarkers for platelet function.

thrombomiR™ kit applications

- **Monitor the drug effects** on platelet function, reactivity and hemostasis
- **Diagnosis of platelet-related disorders**

Unique features of the thrombomiR™ kit:

- **Works with frozen sample material** (serum or plasma)
- **Responds to all platelet-activating signals** (ADP, Collagen, etc.)
- **Measures a platelet-signal generated in-vivo** thus complementing results from ex vivo platelet-function tests (LTAs, VASP, ...)

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Data analysis:

1. RNA extraction
2. Plasma
3. cDNA synthesis
4. qPCR preparation
5. qPCR analysis
6. Data analysis with thrombomiRTM software
7. Stability for life.
How does it work?

**all-in-one RT-qPCR kit**

the thrombomiR™ kit contains all necessary reagents for:

1. RNA extraction
2. cDNA synthesis
3. Preparation of qPCR Mix
4. Real time qPCR analysis
5. Data analysis: proprietary software

Which type of samples can be used?

**Platelet miRNA content in different blood components**

<table>
<thead>
<tr>
<th>Blood Component</th>
<th>miRNA Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>Decreases</td>
</tr>
<tr>
<td>Serum</td>
<td>During coagulation</td>
</tr>
<tr>
<td>Conventional Plasma</td>
<td>Residual platelets, Platelet activation during preparation</td>
</tr>
<tr>
<td>PPP</td>
<td>Baseline miRNA release</td>
</tr>
</tbody>
</table>

The thrombomiR™ test should be used with platelet-poor plasma. Alternatively, serum can be used if coagulation time has been kept constant. Visit our website at www.tamirna.com/sample-requirements for further information.
Circulating microRNAs are a novel class of blood-borne biomarkers. They are secreted from virtually any cell in the human body and distributed to the circulation. Local pathophysiology in tissues can be detected using other cells via the circulation. They are stable for life.

Unique features of the thrombomiR™ kit:

- Diagnosis of biomarkers for platelet function.
- Measures a platelet-signal generated in vivo, thus complementing results from ex vivo platelet-function tests (LTAs, VASP, ...).
- Responds to all platelet-activating signals (ADP, Collagen, ...).
- Works with frozen sample material.
- All-in-one RT-qPCR kit.
- One kit allows analysis of up to 48 samples.
- High throughput: one kit allows analysis of up to 48 samples (6 samples/plate, 8 plates/kit).
- Fast and simple data analysis: thrombomiR™ software included.

### microRNAs included in the thrombomiR™ kit

<table>
<thead>
<tr>
<th>miRNA ID</th>
<th>platelet enrichment</th>
<th>platelet function</th>
<th>other cardiovascular functions</th>
<th>main cellular origin in plasma</th>
<th>validated pathways/targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-126-3p</td>
<td>+++</td>
<td>platelet activation</td>
<td></td>
<td>platelets, megakaryocytes &amp; endothelial cells</td>
<td>VEGF signaling: SPRED1 and PDK3/p85-β</td>
</tr>
<tr>
<td>hsa-miR-223-3p</td>
<td>+++</td>
<td>aggregation and granularity secretion</td>
<td></td>
<td>platelets &amp; megakaryocytes</td>
<td>P2Y12 receptor J</td>
</tr>
<tr>
<td>hsa-miR-197-3p</td>
<td>+++</td>
<td>platelet activation</td>
<td></td>
<td>platelets</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-191-5p</td>
<td>+++</td>
<td>platelet activation</td>
<td></td>
<td>platelets &amp; endothelial cells</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-24-3p</td>
<td>++</td>
<td>platelet activation</td>
<td></td>
<td>platelets &amp; endothelial cells, monocytes</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-21-5p</td>
<td>++</td>
<td>platelet biogenesis</td>
<td></td>
<td>platelets &amp; hematopoietic cells</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-28-3p</td>
<td>++</td>
<td>megakaryocyte differentiation +</td>
<td>inhibits cell growth in VSMCs</td>
<td>vascular smooth muscle cells, endothelial cells, cardiac fibroblasts, and cardiomyocytes, platelets</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-320a</td>
<td>++</td>
<td>platelet activation</td>
<td>insulin signaling, angiogenesis, progression of retinopathy</td>
<td>platelets &amp; endothelial cells</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-150-5p</td>
<td>+</td>
<td>platelet activation, megakaryocytepoiesis</td>
<td>insulin signaling, angiogenesis</td>
<td>leukocytes, megakaryocytes &amp; monocytes</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-27b-3p</td>
<td>+</td>
<td>megakaryocyte differentiation</td>
<td>angiogenesis, vascular disease and vascular aging, progression of retinopathy</td>
<td>platelets &amp; vasculature</td>
<td></td>
</tr>
<tr>
<td>hsa-miR-122-5p</td>
<td>–</td>
<td></td>
<td>Fatty acid and cholesterol synthesis in hepatocytes</td>
<td>liver tissue</td>
<td>multiple genes required for hepatocyte differentiation and fatty acid synthesis</td>
</tr>
</tbody>
</table>

The publications list leading to the identification of these novel biomarker candidates can be found on our homepage: [www.tamirna.com/products/thrombomir.html](http://www.tamirna.com/products/thrombomir.html)

### Key publications

Clinical utility of the thrombomiR™ kit

- Platelet microRNAs are released from cells upon activation.
- Release is independent of the activation pathway (e.g. ADP, collagen, etc.).
- MicroRNAs are protected from degradation in serum/plasma due to vesicular encapsulation.

Correlation between ex vivo platelet aggregation tests and platelet-derived microRNAs.

Correlation between microRNA levels and results of VerifyNow test (A) and the VASP assay (B) in patients on dual antiplatelet therapy for 30 days post acute coronary syndrome. PRU denotes P2Y12 reaction units (y axis). Higher PRU values reflect higher P2Y12-mediated platelet reactivity.


Low levels of thrombomiRs in serum are associated with lower risk of cardiovascular death.

High baseline levels (“upper third”) of miR-197 and miR-223 are associated with reduced survival (due to cardiovascular death) in a cohort of 873 patients, of which 340 are cases with acute coronary syndrome and 533 cases of stable angina pectoris.

From Schulte, C., et al. PLoS ONE, 10(12), pp. 1–12., Figure 1
A simple and standardized kit for qPCR analysis of circulating platelet-derived microRNAs. Low levels of thrombomiRs in serum are associated with lower risk of cardiovascular death. Correlation between ex vivo platelet aggregation tests and platelet-derived microRNAs. Clinical utility of the thrombomiR™ kit.

High baseline levels (“upper third”) of miR-197 and miR-223 are associated with reduced survival (due to cardiovascular death) in a cohort of 873 patients, of which 340 are cases with acute coronary syndrome and 533 cases of stable angina pectoris.


Correlation between microRNA levels and results of VerifyNow test (A) and the VASP assay (B) in patients on dual antiplatelet therapy for 30 days post acute coronary syndrome. PRU denotes P2Y12 reaction units (y axis). Higher PRU values reflect higher P2Y12-mediated platelet reactivity.


MicroRNA Biomarkers of Platelet Function

- Platelet microRNAs are released from cells upon activation.
- Release is independent of the activation pathway (e.g. ADP, collagen, etc.).
- MicroRNAs are protected from degradation in serum/plasma due to vesicular encapsulation.