a simple and standardized kit
qPCR analysis of circulating platelet-derived microRNAs

Neurodegenerative Diseases
Musculoskeletal Diseases
Cardiovascular Diseases

from Schulte, C., et al. PLoS ONE, 10(12), pp. 1–12., Figure 1

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


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.

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

thrombomiiR™ kit applications

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

Unique features of the thrombomiiR™ 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, ...)

Key publications

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>Leukocyte contamination</td>
</tr>
<tr>
<td>Serum</td>
<td>miRNA release 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-requi- rements](http://www.tamirna.com/sample-requirements) for further information.
Assay format

- **Low sample volume**: 200 µL human plasma/serum
- **Platelet function analysis based on the thrombomiR™ signature**: 10 thrombomiRs™ and 6 controls/sample
- **Reduced hands-on time**: primer coated 96 or 384 well plates
- **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

| miRNA ID     | platelet enrichment | platelet function         | other cardiovascular functions                                      | main cellular origin in plasma                                      | validated pathways/targets                                      |
|--------------|---------------------|---------------------------|---------------------------------------------------------------------|---------------------------------------------------------------------|
| hsa-miR-126-3p | +++                 | platelet activation       |                                                                     | platelets, megakaryocytes & endothelial cells                       | VEGF signaling: SPRED1 and PIK3R2/p85-αβ                        |
| hsa-miR-223-3p | +++                 | aggregation and granule secretion |                                                                     | platelets & megakaryocytes                                          | P2Y12 receptor ↓ RPS6KB1/HIF-1α signaling pathway               |
| hsa-miR-197-3p | +++                 | platelet activation       |                                                                     | platelets                                                           |
| hsa-miR-191-5p | +++                 | platelet activation       |                                                                     | platelets & endothelial cells                                      |
| hsa-miR-24-3p  | ++                  | platelet activation       | monocyte differentiation                                             | platelets & endothelial cells, monocytes                            |
| hsa-miR-21-5p  | ++                  | platelet biogenesis       | inhibits cell growth in VSMCs                                       | vascular smooth muscle cells, endothelial cells, cardiac fibroblasts, and cardiomyocytes, platelets |
| hsa-miR-28-3p  | ++                  | megakaryocyte differentiation |                                                                     | platelets & hematopoietic cells                                   |
| hsa-miR-320a   | ++                  | platelet activation, megakaryocyte differentiation | insulin signaling, angiogenesis, progression of retinopathy | platelets & endothelial cells                                      |
| hsa-miR-150-5p | +                   | platelet activation, megakaryocyte differentiation | insulin signaling, angiogenesis                                      | leukocytes, megakaryocytes & monocytes                             |
| hsa-miR-27b-3p | +                   | megakaryocyte differentiation | angiogenesis, vascular disease and vascular aging, progression of retinopathy | platelets & vasculature                                              |
| hsa-miR-122-5p | –                   |                             |                                                                      | liver tissue                                                       |

The publications list leading to the identification of these novel biomarker candidates can be found on our homepage: 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.

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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.

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