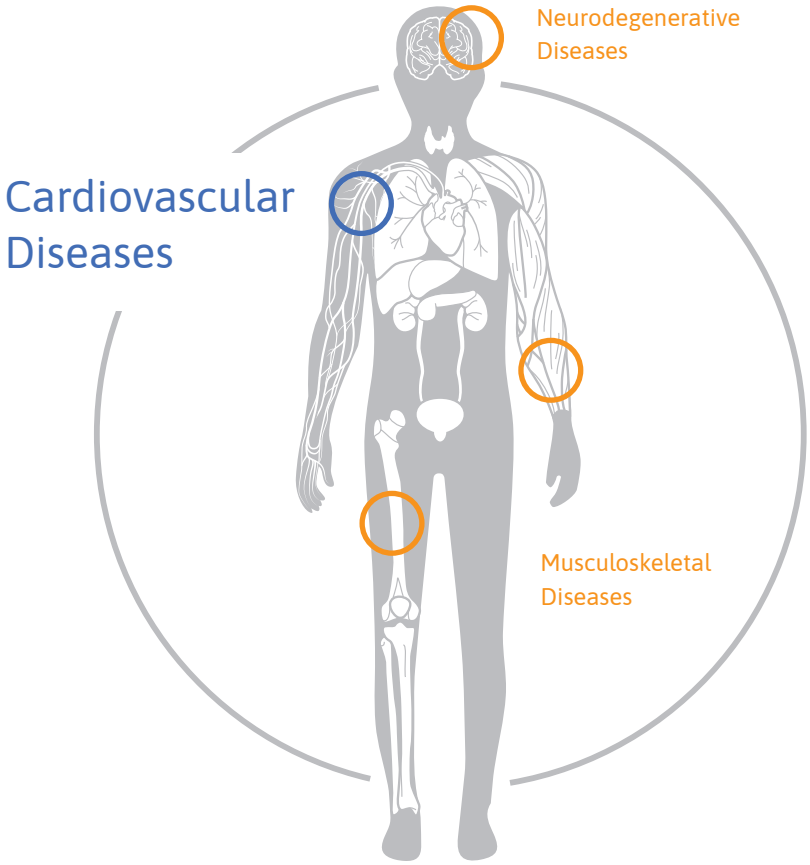
thrombomiR™

microRNA Biomarkers of Platelet Function



a simple and standardized kit

qPCR analysis of circulating platelet-derived microRNAs

TAmiRNA

stability for life.

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

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.

thrombomiR™ kit applications

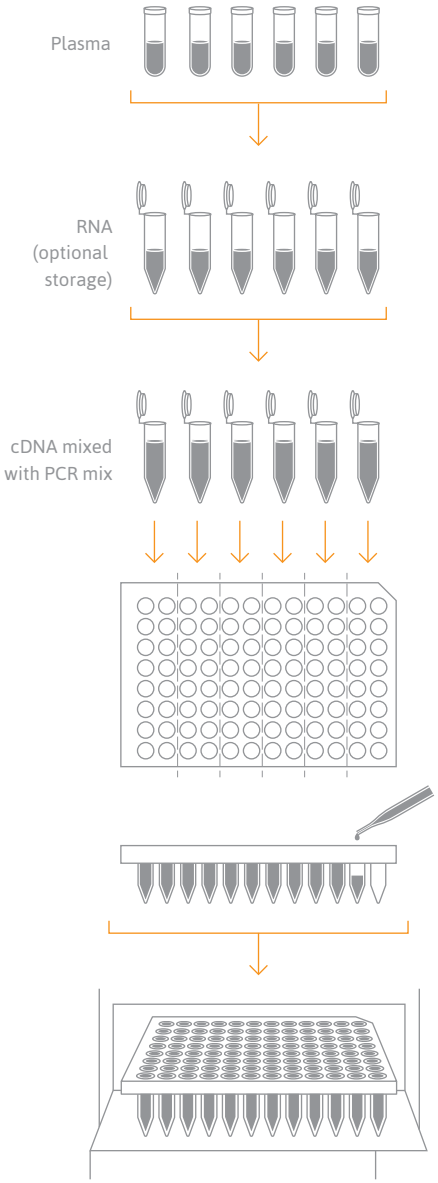
- **Monitor the drug effects** on platelet function, reactivity and hemostasis
- Diagnosis of **platelet-related disorders**

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, ...)

How does it work?

all-in-one RT-qPCR kit
the thrombomiR™ kit contains all necessary reagents for:



1 RNA extraction

2 cDNA synthesis

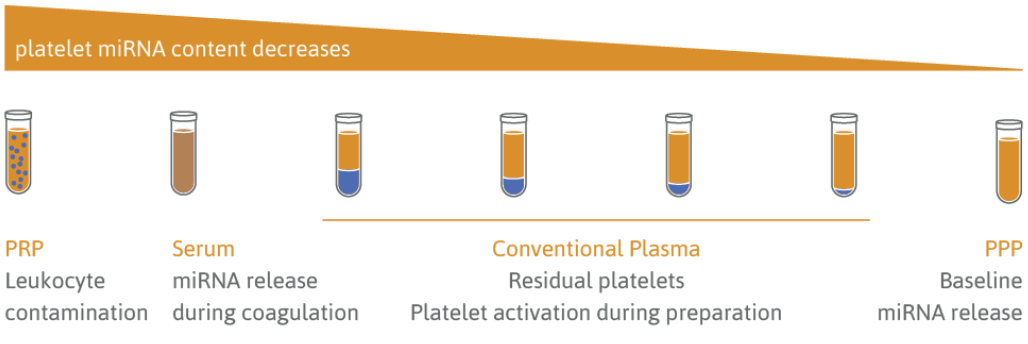
3 Preparation of qPCR Mix

4 Real time qPCR analysis

5 Data analysis: proprietary software

Which type of samples can be used?

Platelet miRNA content in different blood components



PRP
Leukocyte contamination

Serum
miRNA release during coagulation

Conventional Plasma
Residual platelets
Platelet activation during preparation

PPP
Baseline miRNA release

The thromobmiR™ 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

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-β ↓ Vascular inflammatory pathways: VCAM-1 ↓
hsa-miR-223-3p	+++	aggregation and granule secretion		platelets & megakaryocytes	P2Y12 receptor ↓ RPS6KB1/HIF-1a 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 differentation	platelets & endothelial cells, monocytes	PDGF-BB signaling: GATA2, PAK4 ↓: Vascularity, cardiac function, and infarct size after myocardial infarction
hsa-miR-21-5p	++	platelet biogenesis	inhibits cell growth in VSMCs	vascular smooth muscle cells, endothelial cells, cardiac fibroblasts, and cardiomyocytes, platelets	PTEN, BMPR2, WWP1, WASp
hsa-miR-28-3p	++	megakaryocyte differentiation ↓		platelets & hematopoietic cells	
hsa-miR-320a	++		insulin signaling, angiogenesis, progression of retinopathy	platelets & endothelial cells	Survivin, VEGF
hsa-miR-150-5p	+	platelet activation, megakaryocyto-poiesis ↑	insulin signaling, angiogenesis	leukocytes, megakaryocytes & monocytes	c-Myb, VEGF-a, HIF-1a
hsa-miR-27b-3p	+	megakaryocyte differentiation	angiogenesis, vascular disease and vascular aging, progression of retinopathy	platelets & vasculature	PPARγ, SMAD7
hsa-miR-122-5p	–		fatty acid and cholesterol synthesis in hepatocytes	liver tissue	multiple genes required for hepatocyte differentiation and fatty acid synthesis

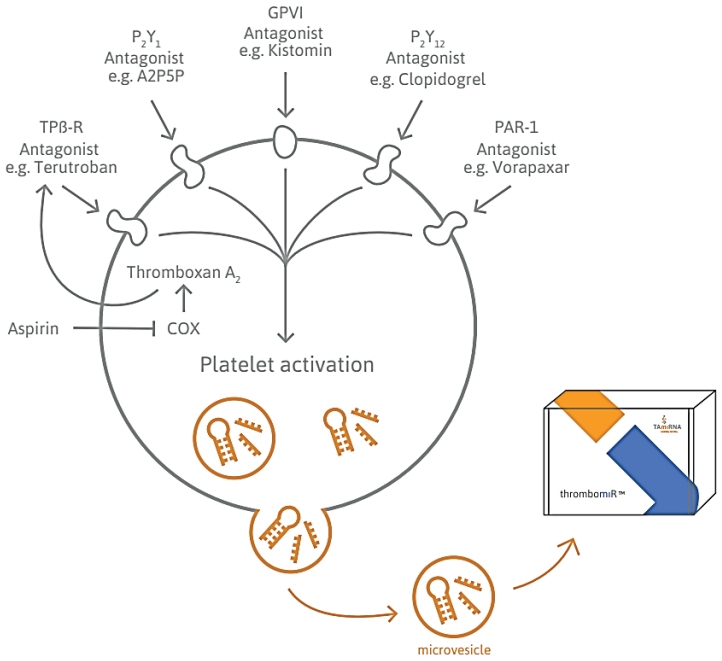
The publications list leading to the identification of these novel biomarker candidates can be found on our homepage: www.tamirna.com/products/thrombomir.html

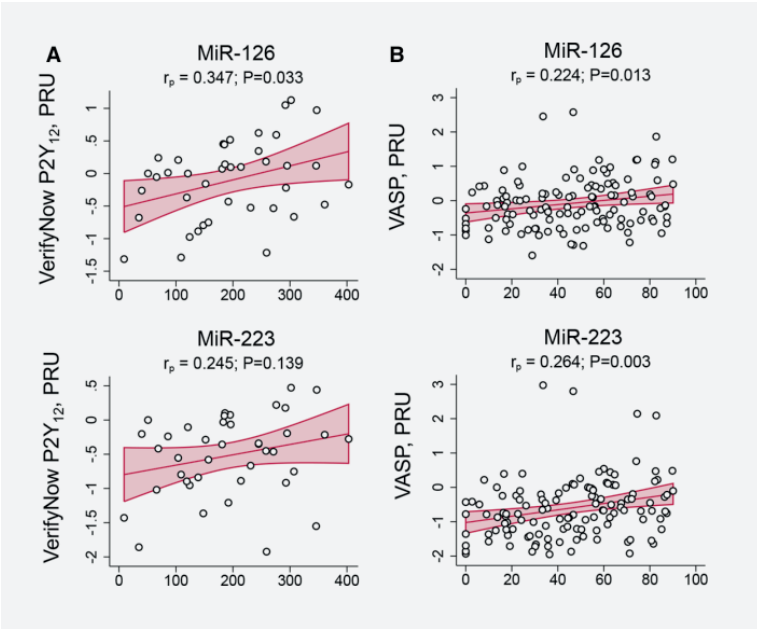
Key publications

1. Bye A, et al. Circulating microRNAs predict future fatal myocardial infarction in healthy individuals - The HUNT study. 2016 J Mol Cell Cardiol.
2. Kaudewitz D, et al. Association of MicroRNAs and YRNAs With Platelet Function. 2016 Circ Res.
3. Mayr M, et al. MicroRNAs within the continuum of postgenomics biomarker discovery. 2013 Arterioscler Thromb Vasc Biol.
4. Willeit P, et al. Circulating microRNAs as novel biomarkers for platelet activation. 2013 Circ Res.
5. Willeit P, et al. Circulating MicroRNA-122 Is Associated With The Risk of New-Onset Metabolic Syndrome And Type-2-Diabetes. 2016 Diabetes.
6. Zampetaki A, et al. Prospective study on circulating MicroRNAs and risk of myocardial infarction. 2012 J Am Coll Cardiol.
7. Zampetaki A, et al. Plasma microRNA profiling reveals loss of endothelial miR-126 and other microRNAs in type 2 diabetes. 2010 Circ Res.
8. Sunderland N, et al. MicroRNA Biomarkers and Platelet Reactivity: The Clot Thickens. 2017 Circ. Res.

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.

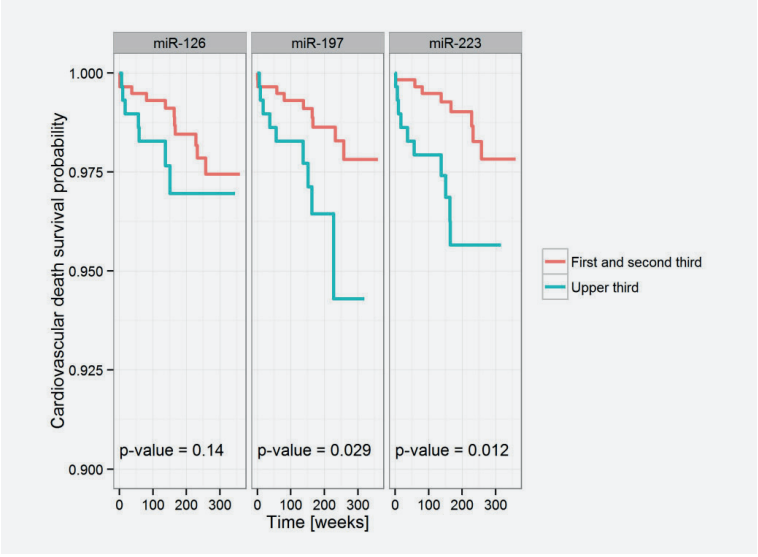




Correlation between ex vivo platelet aggregation tests and platelet-derived microRNAs.

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.

From Sunderland N, et al. MicroRNA Biomarkers and Platelet Reactivity: The Clot Thickens. 2017 Circ. Res., Figure 6.



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

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TAmiRNA

stability for life.

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