Standardization procedure for plasma biomarker analysis in rat models of epileptogenesis: Focus on circulating microRNAs

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Supplementary table 1. Summary of blood sampling protocols in studies using rat plasma for miRNA detection

Disease or phenomena	Sampling site	Blood collection	Plasma separation/ storage	Plasma QC	Plasma volume/RNA extraction	RNA QC /spike-ins	miRNA platform	Validation	Normalization / method	Calibrator/ other controls	Ref
Acute kidney injury	Abdominal aorta	EDTA			miRcute, mirVana without phenol	Bioanalyzer 2100	GoScript with stemloop primers		miR-16/ 2^-ΔΔCT		1
Non-identical miRNA profiles between arterial and venous plasma	abdominal aorta or vena cava	EDTA + RNAase inhibitor	1600 g, 10 min; 16,000 g, 4°C, filtering 0.22 µm filter		miRNeasy		Agilent Rat miRNA V21.0 Microarray	RT-qPCR	Cel-miR-39-3p/ 2^-ΔΔCT		2
Diabetic nephropathy	Tail vein				TRIzol	Synthetic oligonucleotide	Hairpin-it miRNA RT-qPCR		U6-snRNA/ 2^-ΔΔCT	miR-130b	3
Chronic sucrose ingestion associated inflammation	`	K+EDTA	3000 rpm,15 min, 4°C/-70°C		100 µl/ miRNeasy, exoRNeasy	Cel-miR-39, bacterial ribosomal RNA	TaqMan RT-qPCR		Cel-miR-39/ 2^-ΔΔCT		4
Type 2 diabetes	Tail vein				150 µl/ miRNeasy	Bioconductor package HTqSeq v1.16.0	Taqman miRNA Array, Card A V.2.0		Global mean normalization		5
Antigen-induced pulmonary inflammation	Abdominal aorta	Citrate	1800 g, 20 min, 4°C/-80°C	Coagulated samples excluded, hemolysis avoided	200 µl/ Tri reagent BD	NanoDrop 2000c /Cel-miR-39 spike-in	iQ5 system		Cel-miR-39/ 2^-ΔΔCT	Non-spike-in control, non-template control, non-RT control, custom control pool	6
Heparinase treatment of	Aorta	EDTA, < 30	1600 g, 4°C,		200 µl/ TRIzol	Bioanalyzer 2100 /E. coli	TaqMan RT-		Absolute quantification		7

heparin-contaminated plasma		min	2x/-80°C		LS	tRNA, cel-miR-39-3p	qPCR		using calibration curves from synthetic miRNAs		
CNS impairments associated with Gulf War Illness	Cardiac puncture or right atrium	EDTA	1900 g, 10 min, 4°C; 16,000 g, 10 min / -80° C; 16,000 g, 5 min, 4°C		200 µl/ miRNeasy, ExoQuick	Bioanalyzer 2100/ Cel-miR-39, synthetic control miRNA	miScript RT-qPCR	Illumina HiSeq2000	Cel-miR-39/ ΔΔCT		8
Acute rejection post limb transplantation	Caudal vein				100 µl/ mirVana PARIS		TaqMan RT-qPCR		Synthetic ath-mir-159a/ ΔΔCT		9
Acute skeletal muscle damage	Exsanguination	K_2-EDTA	2000 g, 10 min, 4°C, 2x		100 µl/ mirVana PARIS	miR-103a-3p	Mouse&Rat panel IþII,V3.M (Exiqon), Customized qPCR plates, Pick-&-Mix microRNA	miScript RT-qPCR	Geometric mean of several miRNAs, based on geNorm algorithm	Plasma pools enriched with RBC and platelet pellets to study effects of RBC and platelet enrichment.	10
Contrast-induced acute kidney injury		EDTA, < 1 hour, RT	820 g, 10 min, 4°C; 16,000 g, 10 min, 4°C / -80°C		mirVana	NanoDrop 1000 / cel-miR-39	Agilent microRNAs microarray v.10.1	TaqMan RT-qPCR	Cel-miR-39/ 2^-ΔΔCT		11
Fibrotic liver disease			3000 rpm, 10 min, 4°C/ -80°C		miRNeasy	Cel-miR-39	TaqMan RT-qPCR		U6-snRNA/ 2^-ΔΔCT		12

Nephrotoxicity and hepatotoxicity	Lateral tail vein (non-terminal) or vena cava (terminal)	EDTA	4°C		200 µl/ Asuragen	RT-qPCR of 3 miRNAs	miR-seq using TruSeq Small RNA Library Kit		U6-snRNA/ ΔΔCT		13
Acute kidney injury	Abdominal aorta	EDTA, < 1 hour	3000 g, 15 min, 4°C; 12,000 g, 15 min, 4°C		mirVana PARIS	Cel-miR-39	rodent TaqMan low-density array assay	TaqMan RT-qPCR	Cel-miR-39/ 2^-ΔΔCT		14
Comparison of three pulsed focused ultrasound regimes to release tumor-derived miRNA into circulation	Jugular vein via implanted polyurethane catheters for repeated sampling	K_2-EDTA gentle inversion of blood samples 20 times without shaking to avoid hemolysis	1300 g, 15 min, RT; 250 µL of plasma aspirated and immediately combined with 1.25 mL of Qiazol, lysed/ -80°C		250 µL/ miRNeasy	Cel-miR-39	Human panel I, V2.M RT-qPCR arrays (Exiqon) using miRCURY LNA Universal RT miRNA PCR kit (Exiqon)	TaqMan RT-qPCR,	Cel-miR-39		15
Diabetic nephropathy	Tail vein				TRIzol		miRScript RT-qPCR	Taqman RT-qPCR using the Hairpin-it miRNA qPCR kit	U6-snRNA / 2^-ΔΔCT		16
Retinal toxicity	Tail vein	EDTA	1500 g, 15 min		100 µL/ miRNeasy	Cel-miR-39	Poly(A) tailing kit, MultiScribe, RT-qPCR		Cel-miR-39, miR-92, miR-181, miR-192		17
Cardiac injury	Abdominal aorta	EDTA tubes pre-cooled on ice, Rapidly centrifuged	2100 g, 10 min, 4°C/ -80°C		200 µL/ miRNeasy	Ath-miR-159a	TaqMan customized multiplexed miRNA assay	ath-miR159a	Calibration curves from pooled synthetic miRNA stocks, plus miR-16-15p and mR-192-5p		18

Perinatal glutamate exposure	Atrial cathethers for repeated sampling and trunk blood				miRNeasy	260/280 ratio	miScript II, miRNA qPCR assay using Rat miFinder RT2 miRNA PCR 96-well plate array		6 normalization controls, and controls to assess recovery, RT and PCR performance/ ΔΔCT		19
Drug-induced cardiac injury	Posterior vena cava	Li-heparin S-Monovette tubes		miR-451, miR-486	40 µL/ Firefly Bioworks		high throughputmiR-profiling technology from Firefly BioWorks		miR-92a-3p, miR-103-3p, miR-106b-5p		20
Contrast-induced acute kidney injury	Tail cutting				miRNeasy	Cel-miR-39	miScript RT-qPCR		Cel-miR-39/ 2^-ΔΔCT		21
Third-degree burn	Abdominal aorta	Lithium heparin	200 g, 10 min/ -80°C		TRIzol	TRIzol	Revert Aid, SsoFast EvaGreen RT-qPCR		U6-snRNA		22
Temporal lobe epilepsy	Intracardiac withdrawal	EDTA	1000 g, 2min; 2500 g, 2 min/ -80°C		200 µL/ miRNeasy	Cel-miR-39	Rat miRNA MicroArray Kit, Human micro-RNA Microarray V3, #G4470C, Agilent	TaqMan RT-qPCR	Cel-miR-39/ Δ CT		23
Pulmonary arterial hypertension	Inferior vena cava	EDTA	1700 g, 10 min, 4°C; 11,000 g, 2 min, 4°C/ -80°C; 11,000 g, 5 min, 4°C		100 µL/ miRNeasy	Cel-miR-39	miScript II RT-qPCR		miR-24-3p/ Δ CT		24

Roux-en-Y gastric bypass surgery	Cardiac puncture	EDTA, immediately	3500 g, 10 min, RT/ -80°C		100 µL/ mirVana PARS	Cel-miR-39	TaqMan RT-qPCR	Taqman miRNA assay	U6-snRNA, Cel-miR39, mean Ct from 5 stable miRNAs, median Ct based global normalization		25
Retinopathy of prematurity	Orbital blood				miRNeasy	Qubit 2.0 Fluorometer, NanoDrop 1000	Illumina HiSeq 2500	RT-qPCR	U6-snRNA		26
Drug-induced cardiotoxicity	Tail vein	K_2-EDTA	1500 g, 15 min, refrigeration/ -80°C		100 µL/ miRNeasy		TaqMan RT-qPCR		U6-snRNA/ comparative method	Negative control samples	27
Anesthesia induced regulation of muscle specific miRNAs	Inferior vena cava	EDTA	1700 g, 15 min, 4˚C/ -80°C		Isogen-LS		TaqMan MicroRNA Assay Rodent Panels (A and B, v.3.0)	TaqMan RT-qPCR	Cel-miR-39, Cel-miR-238/ Δ CT	A sample in the control group was arbitrarily designated as the calibrator	28
Renal ischemia-reperfusion injury	Tail-cutting				200 µL/ TRIzol, miRcute	Mmu-miR-295	miScript RT-qPCR		Mmu-miR-295		29
Effects of laparotomy and anesthesia on hepatic miRNA expression	Aorta	EDTA, < 30 min	1500 g, 15 min/ -80°C; 12,000 g, 30 min, 4˚C		50 µL/ TRIzol	Bioanalyzer 2100	TaqMan RT-qPCR		miR-451		30
Hepatocellular injury		EDTA									31

Temporal lobe epileptogenesis	Trunk blood	K_2-EDTA, immediately	1500 g, 10 min, 4˚C/ -80°C		200 µL/ miRNeasy	Bioanalyzer 2100	TaqMan RT-qPCR		miR-23a/ 2^-ΔΔCT		32
Hypertension-induced heart failure	Catheters implanted in left femoral artery for repeated sampling. Sacrifice blood from vena cava	EDTA	1500 g, 15 min, 4˚C/ -80°C		100 µL/ TRIzol LS	Cel-miR-2, Cel-lin-4	TaqMan Array miRNA rodent card A	TaqMan RT-qPCR	Cel-miR-2, Cel-lin-4		33
Classification of muscular tissue	Inferior vena cava	EDTA	1600 g, 15 min, 4°C		mirVana PARIS		ABI TaqMan Rodent MicroRNA Array kit (Card A and B)	TaqMan RT-qPCR	Synthethic small RNA		34
Acute phased pancreatic injury	Inferior vena cava	EDTA	1600 g, 15 min, 4°C		mirVana PARIS				Synthethic small RNA		34
Congestive Heart Failure	Inferior vena cava	EDTA	1600 g, 15 min, 4°C		mirVana PARIS		ABI TaqMan Rodent MicroRNA Array kit (Card A)	TaqMan RT-qPCR	Synthethic small RNA		34
Drug-induced ocular toxicity	Exsanguination	EDTA			100 µL/ miRNeasy		Poly(A) tailing kit, MultiScribe, RT-qPCR		miR-192		35
Drug-induced hepatotoxicity	Sublingual veins				200 µL/ miRNeasy	Bioanalyzer 2100/ ath miRNA control	TaqMan RT-qPCR		ath miRNA control/ ΔCT		36
Acetaminophen-induced hepatotoxicity		Gel and clot activator tube	415 g, 5 min, 4°C		TRIzol, mirVana		miScript RT-qPCR		miR-16, miR-92a, let-7a, miR-103, 5S-snRNA, U6-snRNA using		37

									geNorm, BestKeeper, Normfinder/ ΔCT		
Hepatocellular Injury, Cholestasis, and Steatosis		EDTA, 30 min on ice	?/ -80°C		600 µL/ mirVana PARIS	Cel-miR-39	TaqMan Array Rodent MicroRNA A+B Cards Sets v2.0		3 endogenous controls/ ΔCT		38
Hypertension-induced heart failure					TRIzol LS	Cel-miR-2, Cel-lin-4	TaqMan RT-qPCR		Cel-miR-2, Cel-lin-4		39
Cerebral infarction	Right or left jugular vein	EDTA	1600 g, 15 min, RT		mirVana PARIS	miR-124	TaqMan RT-qPCR		miR-124	miR-451, 5S-snRNA	40
Pancreatic injury	Caval vein	K_2-EDTA, 30 min-2 h, RT	1200 g, 20 min, 4°C		100 µL/ TRI Reagent BD	Cel-miR-39	TaqMan RT-qPCR		Cel-miR-39		41
Middle cerebral artery occlusion	Vena cava	EDTA	±2000 g, 20 min, 4 °C/ -70°C		TRIzol	NanoDrop 1000	TaqMan RT-qPCR		Calibration curves of control miRNA diluted into yeast RNA		42
Myocardial Injury	Tail vein	EDTA	?/ -70°C		200 µL/ mirVana PARIS	^{32}P-miR208	TaqMan RT-qPCR		miR-208/ 2^(35-Ct)	5S-snRNA	43

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