

RNA isolation <i>BioVendor</i>		hsa-miR-145-5p hsa-miR-1-3p hsa-miR-423-5p <i>BioVendor</i>	
Prepare samples in tubes	+ 300µL serum	Hybridization in nuclease-free PCR tube	+ 20µL standards, Quality Control, samples
	+ 90µL Lysis Buffer		
	Vortex 5 s.		+ 20µL DNA Probe working solution
	Incubate 3 min. RT		
Precipitate protein	+ 30µL Protein Precipitation Buffer		Incubate 3 min. 85°C
	Vortex 5 s.		
	Incubate 1 min. RT		Incubate 2 min. 4°C
	Centrifuge 3 min. 11000xg		
Transfer supernatant to Collection Tube (2 mL)			Incubate 5 min. 37°C
Adjust binding cond.	+ 400µL isopropanol	miREIA procedure in microplate	
	Vortex 5 s.		+ 90µL diluted hybrids
Bind RNA & DNA	Load sample on isolation column		
	Incubate 2 min. RT		Incubate 1h 37°C
	Centrifuge 30 s. 11000xg		
Digest DNA	+ 700µL Wash Buffer		Wash 5x 350µL Wash Solution
	Centrifuge 30 s. 11000xg		
	+ 250µL Wash Buffer		+ 100µL Streptavidin-HRP Conjugate
	Centrifuge 2 min. 11000xg		
	+ 50µL rDNase in Reaction Buffer		Incubate 30 min. 37°C
Wash and dry	Incubate 15 min. RT		
	100µL Wash Buffer		Wash 5x 350µL Wash Solution
	Centrifuge 30 s. 11000xg		
	700µL Wash Buffer		+ 100µL Substrate Solution
	Centrifuge 30 s. 11000xg		
Elute RNA into a new Collection Tube (1.5 mL)	250µL Wash Buffer		Incubate 15 min. RT
	Centrifuge 2 min. 11000xg		
	+ 30µL RNase-free H ₂ O		+ 100µL Stop Solution
	Incubate 1 min. RT		
	Centrifuge 1 min. 11000xg		Read absorbance 450 nm within 5 min.
Freeze isolate sample -20°C			

	STEP	PCSK9 MyBPC3 <i>BioVendor</i>	Anty-PR3 Anty-MPO <i>EUROIMMUN</i>	NOX1 CYBb NCF2 DNASE1 <i>Cloud-Clone Corp.</i>
	antigen-coated wells	✓	✓	✓
	+ standards/calibrators, controls, samples, blank	100µL	100µL	100µL
	incubation	1h RT shaking	30 min. RT	1 h 37°C
	liquid removed	-	-	✓
	wash	350µL Wash Solution 5x	-	-
	+	100µL Biotin Labelled Antibody	-	100µL Detection Reagent A
	incubation	1h RT shaking	-	1 h 37°C
	wash	350µL Wash Solution 5x	300µL Wash Solution 3x	350µL Wash Solution 3x
	+	100µL Streptavidin-HRP Conjugate	100µL Enzyme conjugate	100µL Detection Reagent B
	incubation	30 min. RT shaking	30 min. RT	30 min. 37°C
	wash	350µL Wash Solution 5x	300µL Wash Solution 3x	350µL Wash Solution 5x
	+ Substrate Solution	100µL	100µL	90µL protect from light
	incubation	15 or 10 min. RT	15 min. RT	20 min. 37°C
	+ Stop Solution	100µL	100µL	50µL
	measurement	450nm within 5 min.	450nm	450nm