

# **ID3EAL™ miRNA Knowledge Panels**

MiRXES ID3EAL<sup>™</sup> miRNA Knowledge Panels are ready-to-use assay panels for interrogating miRNAs relevant to specific areas of research. Each panel profiles a curated array of disease related miRNAs selected through extensive survey of published literature and in-house research data. All miRNA assays have been extensively wet-lab validated using synthetic miRNA templates and human RNA sample.

MiRXES offers three Knowledge Panels for miRNA research into disease mechanisms, biofluids and cancers. Each kit is formatted to include all necessary reagents for the RNA-to-CT workflow. Unique synthetic templates are built into the assays to monitor and normalize technical variations from RNA isolation to qPCR. These kits are compatible with major real-time qPCR platforms and are available in various pack sizes.



#### PanoramiR miRNA Panel

The ID3EAL<sup>™</sup> PanoramiR Knowledge Panel is a thoughtfully designed and robust platform for miRNA diagnostic biomarker discovery, disease subtyping and miRNA molecular phenotyping.

Targeting 376 miRNAs expertly curated by MiRXES scientists from miRBase 22, HMDD 3.2, The Cancer Genome Atlas, high-impact publications and in-house research data, the PanoramiR Knowledge Panel allow researchers to quickly gain insights into complex disease mechanisms involving thousands of miRNA-regulated genes spanning 46 different biological pathways.



#### **Biofluid miRNA Panel**

miRNAs are selectively and actively secreted by cells as a means of intercellular communication. These circulating miRNAs have shown extraordinary stability in biofluids and hold great potential as novel non-invasive biomarkers for disease diagnosis, prognosis and monitoring.

The ID3EAL<sup>™</sup> Biofluid Knowledge Panel detects for 176 circulating miRNAs selected from in-house research of more than 20,000 human serum, plasma and urine samples in both healthy and pathological conditions. These well-annotated miRNAs are associated with diverse pathways such as cell signaling, transcriptional and translational regulation, viral infection and many more.



#### **Cancer miRNA Panel**

miRNAs are intimately involved in every hallmark of cancer. Detecting miRNA dysregulations could provide insights into cancer pathogenesis, drug response and recurrence.

The ID3EAL<sup>™</sup> Cancer Knowledge Panel detects for 352 miRNAs that are strongly associated with onco- and tumor suppressor genes and pathways involved in various types of cancers.

# **Ultrasensitive and Robust miRNA Detection**

Achieve unparalleled sensitivity and specificity with wet-lab validated sequence-specific ID3EAL<sup>™</sup> RT and qPCR forward and reverse primers.



All three (3) sequence-specific ID3EAL™ primers are optimally designed using MiRXES' proprietary algorithm and wet-lab validated with synthetic miRNA templates and RNA from biological samples. Manufactured in ISO 13485 facility.



Figure 1. RNA-to-Ct in 2 hours. Each panel is incorporated with sample processing controls, experimental controls and inter-plate calibrators to ensure reproducibility of data.

#### **RNA to Results in 2 hours**

Using MiRXES' patented miRNA RT-qPCR technology, the ID3EAL<sup>™</sup> Knowledge Panels can produce results from RNA samples in just 2 hours or up to 3.5 hours with the pre-amplification step\* to enhance detection with limiting amounts of starting material.

\*Pre-amplification reagents included in ID3EAL™ PanoramiR miRNA Knowledge Panel

#### **Reliable Data**

Assays optimized by MiRXES' proprietary algorithm and wet-lab validated with synthetic miRNA templates and RNA from biological samples

#### **Ready-to-Use Kit**

All reagents, including two spike-in RNAs and interplate PCR calibrators, are provided proportionally to maximize convenience and user experience.

#### **Automated Analysis Template**

An Excel template is provided for easy data quality control, CT value normalization and data visualization. In addition, a PowerShell script is available to simplify identification of miRNA gene targets for Gene Ontology and pathway enrichment analysis.

## Application Note - MiRXES ID3EAL<sup>™</sup> PanoramiR miRNA Panel

ID3EAL<sup>™</sup> PanoramiR reveals differential miRNA expression in mesenchymal stem cells cultured in different cell culture media. Mesenchymal stem cells were grown in two different types of culture media, A and B, to assess the impact of culture media on miRNA expression. miRNAs from the cell pellet (CP) and miRNAs secreted from the cells into the cell culture media (CCM) were profiled in biological triplicates using ID3EAL<sup>™</sup> PanoramiR. 100ng of total RNA isolated from CP and 5ul of RNA isolated from CCM were used for reverse transcription and pre-amplification respectively.

353 and 298 miRNAs out of 376 miRNAs assayed were detected in all CP and CCM samples respectively. Raw Ct values were normalized to spike-in controls and biological normalization using global mean was applied before inter-sample miRNA log<sub>2</sub>FC calculation. miRNA expression differences between groups A and B were visualized through volcano plots and heatmaps. 12 miRNAs were differentially expressed (groups A vs. B) for CP samples and 33 miRNAs for CCM samples. 4 (CP) and 10 (CCM) miRNAs showing statistically significant expression differences (p < 0.05) were identified as candidate miRNAs for further studies.



Figure 4. Data normalization approach. Raw Ct values are normalized to synthetic spike-in controls and subjected to global mean normalization before further data analysis.



Figure 5. Boxplots showing Ct values of miRNAs detected using the ID3EAL<sup>™</sup> PanoramiR miRNA Knowledge Panel. Dotted lines demarcate Ct values 9 and 33. The Ct cut-off of 33 was applied for this analysis where Ct > 33 cycles is considered not detected. (left) 353 out of 376 miRNAs assayed were detected in all CP samples. (right) 298 out of 376 miRNAs assayed were detected in all CCM samples.



Figure 6. Volcano plots showing log<sub>2</sub>FC on the x-axis and p-value significance on the y-axis. (left) 12 miRNAs in CP samples showed expression differences between groups A and B, of which 4 miRNA candidates had statistically significant differences (p<0.05). (right) 33 miRNAs in CCM samples showed expression differences between groups A and B, of which 10 miRNA candidates had statistically significant differences (p<0.05).





Figure 7. Unsupervised clustering of miRNAs with expression differences between groups A and B. Distinct miRNA expression profiles between groups A and B were observed for both CP and CCM samples.



Figure 8. Summary of findings from CP and CCM samples.

## Application Note - MiRXES ID3EAL<sup>™</sup> Biofluid miRNA Panel

MiRXES ID3EAL<sup>TM</sup> Biofluid miRNA Knowledge Panel was compared to equivalent products from two leading vendors using a common pool of human serum RNA. For each product, RNA extracted from 200  $\mu$ L of pooled human serum was used at the start of the respective workflows. Detection cut-offs were determined based on manufacturers' recommendations; Ct  $\leq$  33 was recommended for ID3EAL<sup>TM</sup> Biofluid miRNA Knowledge Panel. Of the three biofluid miRNA products, the ID3EAL<sup>TM</sup> Biofluid miRNA Knowledge Panel showed the highest percentage of miRNA detected (Figure 9), further demonstrating the relevance of its curated content.

Separately, the ID3EAL<sup>™</sup> Biofluid miRNA Knowledge Panel was used to illustrate distinct miRNA expression profiles in normal and gastric cancer patient serum (Figure 10).



Figure 9. Head-to-head comparison of MiRXES ID3EAL™ Biofluid miRNA Knowledge Panel against similar panels in the market. MiRXES technology enables significantly more miRNAs to be detected (74%).



Figure 10.  $\Delta C_{\tau}$  fold-change comparison of miRNAs between gastric cancer serum and pooled human serum. Out of the 130 miRNAs detected, a significant number of miRNAs showed greater than one fold-change (2<sup>1</sup>) expression difference.



# Application Note – MiRXES ID3EAL<sup>™</sup> Cancer miRNA Panel

Four distinct cell types (HDFa, BE2C, HCT116 and U251) were profiled with the ID3EAL<sup>TM</sup> Cancer miRNA Knowledge Panel. One hundred nanograms of total RNA from each sample was used for reverse transcription. Approximately 300 miRNAs were detected for each cell line, with 244 common miRNAs detected with Ct  $\leq$  33 across all four cell lines (Figure 11 & 12). Interplate calibrator normalization and biological normalization using global mean or reference miRNAs were performed before inter-sample miRNA  $\Delta\Delta$ Ct calculation. miRNA expression differences between HFDa and U251 cells were visualized through a scatter plot. Data points that fell outside of ±1 cycle regression lines represented miRNAs that were differentially expressed in both cell lines (Figure 13).



#### Number of miRNAs Detected

Figure 11. Bar chart showing the number of miRNA detected for the four cell lines profiled. Out of the 352 miRNA targets in the panel, 319, 297, 307 and 315 miRNAs were detected from HDFa, BE2C, HCT116 and U251, respectively.



Figure 12. Boxplot showing Ct values of miRNAs detected using the ID3EAL<sup>™</sup> Cancer miRNA Knowledge Panel. The Ct cut-off of 33 was applied for this analysis. At least 84% (i.e., 244 miRNAs) of the miRNAs curated in the panel demonstrated expression relevance.



Figure 13. Scatter plot comparison of miRNA expression differences between U251 and HDFa. One-fold change was applied to this analysis.



# **Purchasing Information**

Item description	Product long description	SKU-UID
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (12)	Qty 2 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 12 samples, 2 x 24 µl of ID3EAL miRNA RT Primers 96-plex, 2 x Panel RNA Spike-in, 24 x Bio-Rad/Roche 96-well PCR plates, UID: HSA02100ABI, Homo sapiens	1105253-HSA02100ABI
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (12)	Qty 2 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 12 samples, 2 x 24 µl of IDEAL miRNA RT Primers 96-plex, 2 x Panel RNA Spike-in, 24 x Abi Fast 96-well PCR plates, UID: HSA02100AFA, Homo sapiens	1105253-HSA02100AFA
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (12)	Qty 2x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 12 samples, 2 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 2 x Panel RNA Spike-in, 24 x Abi Standard 96-well PCR plates, UID: HSA02100AST, Homo sapiens	1105253-HSA02100AST
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (24)	Qty 4 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 24 samples, 4 x 24 µl of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Bio-Rad/Roche 96-well PCR plates, UID: HSA02100ABI, Homo sapiens	1105254-HSA02100ABI
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (24)	Qty 4 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 24 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Abi Fast 96-well PCR plates, UID: HSA02100AFA, Homo sapiens	1105254-HSA02100AFA
ID3EAL Biofluid miRNA Knowledge Panel, 96-well (24)	Qty 4 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 24 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Abi Standard 96-well PCR plates, UID: HSA02100AST, Homo sapiens	1105254-HSA02100AST
ID3EAL Cancer miRNA Knowledge Panel, 96-well (12)	Qty of 4x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 12 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Bio-Rad/Roche 96-well PCR plates, UID: HSA04110BBI, Homo sapiens	1105263-HSA04110BBI
ID3EAL Cancer miRNA Knowledge Panel, 96-well (12)	Qty of 4x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 12 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Abi Fast 96-well PCR plates, UID: HSA04110BFA, Homo sapiens	1105263-HSA04110BFA
ID3EAL Cancer miRNA Knowledge Panel, 96-well (12)	Qty of 4 x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 12 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 48 x Abi Standard 96-well PCR plates, UID: HSA04110BST, Homo sapiens	1105263-HSA04110BST
ID3EAL Cancer miRNA Knowledge Panel, 96-well (24)	Qty of 8x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 24 samples, 8 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 8 x Panel RNA Spike-in, 96 x Bio-Rad/Roche 96-well PCR plates, UID: HSA04110BBI, Homo sapiens	1105264-HSA04110BBI
ID3EAL Cancer miRNA Knowledge Panel, 96-well (24)	Qty of 8 x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 24 samples, 8 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 8 x Panel RNA Spike-in, 96 x Abi Fast 96-well PCR plates, UID: HSA04110BFA, Homo sapiens	1105264-HSA04110BFA
ID3EAL Cancer miRNA Knowledge Panel, 96-well (24)	Qty of 8 x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 24 samples, 8 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 8 x Panel RNA Spike-in, 96 x Abi Standard 96-well PCR plates, UID: HSA04110BST, Homo sapiens	1105264-HSA04110BST
ID3EAL Biofluid miRNA Knowledge Panel, 384-well (12)	Qty 1 x box. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 12 samples, 2 x 24 $\mu$ l of IDEAL miRNA RT Primers 96-plex, 2 x Panel RNA Spike-in, 6 x Bio-Rad 384-well PCR plates, UID: HSA02100ABI, Homo sapiens	1105353-HSA02100ABI
ID3EAL Biofluid miRNA Knowledge Panel, 384-well (12)	Qty 1 x box. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 12 samples, 2x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex. 2 x Panel RNA Spike-in. 6 x Abi Fast/Bio-Rad 384-well PCR plates, UID: HSA02100AFA, Homo sapiens	1105353-HSA02100AFA
ID3EAL Biofluid miRNA Knowledge Panel, 384-well (24)	Qty 2 x boxes. ID3EAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 24 samples, 4 x 24 $\mu$ l of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 12 x Bio-Rad 384-well PCR plates. UID: HSA02100ABI, Homo sapiens	1105354-HSA02100BI
ID3EAL Biofluid miRNA Knowledge Panel, 384-well (24)	Qty 2 x boxes. IDEAL Biofluid A-B miRNA Knowledge Panel 176 Targets for 24 samples. 4 x 24 $\mu l$ of ID3EAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in. 12 x Abi Fast/Bio-Rad 384-well PCR plates, UID: HSA02100AFA, Homo sapiens	1105354-HSA02100AFA
ID3EAL Cancer miRNA Knowledge Panel, 384-well (12)	Qty 2 x boxes. IDEAL Cancer A-D miRNA Knowledge Panel 352 Targets for 12 samples, 4 x 24 µl of IDEAL miRNA RT Primers 96-plex, 4 x Panel RNA Spike-in, 12 x Abi Fast/Bio-Rad 384-well PCR plates, UID: HSA04110BFA, Homo sapiens	1105363-HSA04110BFA
ID3EAL Cancer miRNA Knowledge Panel, 384-well (24)	Qty 4 x boxes. ID3EAL Cancer A-D miRNA Knowledge Panel 352 Targets for 24 samples, 8 x 24 µl of ID3EAL miRNA RT Primers 96-plex, 8 x Panel RNA Spike-in, 24 x Abi Fast/Bio-Rad 384-well PCR plates. UID- HSA04110BFA, Homo saniens	1105364-HSA04110BFA
ID3EAL PanoramiR miRNA Knowledge Panel, 384-well (12)	Qty 2 x boxes. ID3EAL PanoramiR miRNA Knowledge Panel, 376 Targets for 12 samples, RT Buffer (A/B/C/D), AUG Buffer 4x (A1/A2/B1/B2/C1/C2/D1/D2), PCR Buffer 4x. RT Enzyme, PCR Enzyme. Spike-in RNA Template, 12 x Abi Fast/Bio-Rad 384-well PCR plates. Homo sapiens	FGS0003



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