microRNA
MEASUREMENT SERVICE
(MiRXES, miREIA)

SAMPLES

1. RNA isolation IS included in the measurement service.
   Acceptable sample types and minimum volume required per 1 isolation:
   › serum, minimum volume 350 µl
   › plasma, minimum volume 350 µl
   › whole blood, at least one PAXgene tube
   › tissues (stored in RNAlater), minimum amount must be determined for each case separately

2. RNA isolation IS NOT included in the measurement service.
   › Minimum volume needed is 20 µl of total RNA isolate containing small RNA.
   › This amount is sufficient for determination of several targets by qPCR (exact number depends on RNA concentration) or at least two targets by miREIA (exact number depends on the sample type used).

REQUIREMENTS ON SAMPLE IDENTIFICATION AND HANDLING:

› Date of sampling must be indicated
› Identification of samples by a unique number or code
› List of samples without personal data (electronic form is sufficient)
› Brief description of preanalytical phase
› Brief description of isolation procedure (only if isolation was performed by the customer)
› Total RNA concentration
› Standardized procedures for sample collection and storage
› Shipping on dry ice

qPCR

Specifications of the particular study

› Screening – 3 different screening panels are available. Number of targets that can be analyzed is 90 - 352. Usually, samples from the patients suffering from the same diagnosis are pooled and used for the initial screening.
› Individual qPCR assays – targets are selected by the customer; the number of targets is limited by the concentration and volume of RNA isolate. Various measurement options are available (singleplex vs. multiplex etc.)
› Normalization strategy – specification of endogenous and exogenous controls

Important note: Lead time for the chemicals needed for the experiment is 3 weeks.
miREIA

Specifications of the particular study

› Number of targets is limited by the amount of the isolate and sample type used. Maximum number of targets per one isolate:
  · Whole blood – 10 targets
  · Serum, plasma – 3 targets
  · The other sample types – 4 targets
› Normalisation strategy – exogenous control cel-miR-39-3p can be included which has to be decided before the isolation step

FINAL REPORT PROVIDED BY BIOVENDOR:

Isolation:
› Isolation date, name of the technician
› Lot and catalogue number of the kit
› Concentration of total RNA determined by Nanodrop for each sample

qPCR:
› Lot and catalogue number of the kits
› Reverse transcription date, name of the technician
› qPCR date, name of the technician
› Mean Cq values and standard deviation values for replicates for each sample
› Normalization of mean Cq values to an inter-plate control
› Normalization to endogenous and exogenous controls defined for the given study

miREIA:
› Lot and catalogue number of the kits
› Test date, name of the technician
› Raw absorbance data
› Calibration curve
› Concentrations of samples (amol/µl)
› Quality control result
› Normalization to the amount of total RNA
› Normalization to exogenous control cel-miR-39-3p (if it was included in the measurement)

TIME SCHEDULE

<table>
<thead>
<tr>
<th>Activity</th>
<th>Duration</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preparatory phase</td>
<td>8 weeks</td>
<td>Discussion of the requirements, order confirmation, purchasing the material</td>
</tr>
<tr>
<td>Isolation</td>
<td>4 days</td>
<td>100 samples</td>
</tr>
<tr>
<td>qPCR screening (384 targets)</td>
<td>4 days</td>
<td>2 samples</td>
</tr>
<tr>
<td>qPCR measurement – 5 targets</td>
<td>5 days</td>
<td>100 samples</td>
</tr>
<tr>
<td>miREIA – 5 targets</td>
<td>14 days*</td>
<td>80 samples</td>
</tr>
</tbody>
</table>

* if the kit is in our offer; if the kit has to be produced on-request, it is necessary to add approximately 7 weeks per 2 targets

Contact Information

BioVendor – Laboratorni medicina a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420 549 124 185, Fax: +420 549 211 460
E-mail: info@biovendor.com
› www.biovendor.com