

Please fill out the order confirmation completely and correctly to avoid errors and delays and fax it to Miltenyi Biotec GmbH
Fax no. +49 2204 8306 5727.

If you need assistance, contact technical support at macs@miltenyibiotec.de. Please send your samples and a copied order confirmation form to:

Miltenyi Biotec GmbH
 Zentrale Warenannahme
 Genomics Service Department
 Friedrich-Ebert-Straße 68
 51429 Bergisch Gladbach, Germany



1. How many miRXplore™ Microarrays should be used?

Quantity of microarrays _____

2. Which samples are you sending?

Total RNA (µg/µL) _____

Note: We recommend to send 2–4 µg of total RNA. For details please see p. 3, section 1. Total RNA. A minimum of 400 ng of total RNA is required. The average yields of total RNA extracted from different sources can be found on our website.

Cells _____

Tissue _____

Estimated amount of cells/tissue _____

Species _____

How many samples are you sending? _____

Shipment of samples

Frozen on dry ice only.

Note: For sample processing and shipment, please see instructions on page 3.

3. Please specify service type

- miRXplore™ Standard Service** for microarray analysis of individual sample versus control
- miRXplore Universal Reference Service** uses the miRXplore Universal Reference (UR), a defined pool of synthetic microRNAs as a reference for comparison of multiple samples with the control(s). The control is defined by the customer and it is, for example, the unstimulated experimental sample. By calculating the ratio of signals from sample versus UR over the ratio of control versus UR, the resulting so-called re-ratio reflects indirectly the ratio of sample versus control. Thus, the amount of necessary sample material can be reduced if multiple samples need to be compared. The customers can send in their own references, too.

Please specify the reference:

miRXplore Universal Reference (synthetic microRNA pool)

Reference provided by customer: _____

4. Should samples be pooled?

No

Yes Which samples should be pooled? _____

FC 800005



5. Hybridization scheme

Please fill out the hybridization scheme to advise which samples have to be labeled with Cy3- or Cy5-fluorescent dye in the corresponding columns for Cy3 and Cy5. Both fluorochrome-labeled samples will be hybridized against each other. Normally, controls will be Cy3-labeled.

For **miRXplore™ Universal Reference Service**: Please specify the control in the table below with an asterics (*). Normally, the UR—or the reference provided by the customer—will be Cy3-labeled.

Note: Ensure that your storage vessels are labeled carefully and that the markings are identical with the hybridization scheme.

| Microarray experiment | Cy3-labeled sample | Cy5-labeled sample |
|-----------------------|--------------------|--------------------|
| 1 | | |
| 2 | | |
| 3 | | |
| 4 | | |
| 5 | | |
| 6 | | |
| 7 | | |
| 8 | | |
| 9 | | |
| 10 | | |
| 11 | | |
| 12 | | |

6. Order confirmation

Hereby I order for the above listed samples the Microarray Service according to the order no. _____

| | |
|--------------------------------|--|
| Institution / Company | |
| Full name | |
| Department | |
| Name of principal investigator | |
| Adress | |
| Zip code | |
| Town | |
| Country | |
| Phone | |
| E-mail | |
| Fax | |

Please note: As a special service to our customers, excess RNA is stored at Miltenyi Biotec GmbH for three months after completion of Microarray Service. On request, excess RNA shall be sent to the customer for an additional charge.

In case RNA does not pass quality control, the customer can send new samples and will be charged for additional RNA extractions and RNA quality controls.

I/We hereby confirm that the samples submitted to Miltenyi Biotec GmbH for the experiments described above do not contain any infectious material.

Date, signature _____

Instructions for sample processing and shipment

1. Total RNA
2. Quick-frozen cell pellets
3. Quick-frozen tissue samples

The yield of total RNA, which can be extracted from cells or tissue, is highly dependent on the source of the starting material. The required amounts of starting material are a rough estimate. Please make sure to send enough material. Average yields of total RNA extracted from different sources can be found here www.rna-yields.microarray-services.com.

When preparing biological samples, for example, cultured or primary cells, or tissue, work quickly until the sample is either quick-frozen in liquid nitrogen or stored on dry ice.

Note: Use appropriate storage vessel (e.g. 1.5 mL or 2 mL test tubes or 15 mL or 50 mL centrifuge tubes).

Note: For sample shipment, use styrofoam box and make sure that a sufficient amount of either dry ice (min. 5 kg) for flash-frozen samples is included. Contact your carrier for detailed information.

The following guidelines are designed for the protection of RNA integrity and quantity in samples during processing and shipping of (1) total RNA, (2) cells, or (3) tissue.

1. Total RNA

Any RNA extraction method that preserves small RNAs can be used. Please note that standard silica-based kits for total RNA isolation do not preserve small RNAs due to an exclusion size of nucleic acids shorter than 100 nucleotides (nt).

- Make sure that the isolated RNA contains the complete fraction of small RNA. We recommend to send in total RNA samples and to use Tri Reagent® (Sigma-Aldrich, # T9424) or Trizol® (Invitrogen, # 15596-026) for isolation of total RNA.
- For miRXplore Microarray Service an experimental minimum amount of 400 ng of total RNA is required. Optimal results with high sensitivity can be achieved with 2 µg of total RNA. Whenever possible, sending in 4 µg of total RNA is recommended to be used as a backup and for—if necessary—further quality control. If less than 400 ng total RNA is available, do not hesitate to contact technical support for further info (macstec@miltenyibiotec.de).
- Ship samples on dry ice to Miltenyi Biotec.

2. Quick-frozen cell pellets

Adherent cells

1. Remove cell culture medium by aspiration and wash adherent cells once with cold PBS. Detach cells with trypsin and/or EDTA, stop trypsinization by adding medium, and transfer samples to a tube.
2. Pellet the cells by centrifuging at an appropriate centrifugal force such as 100–500×g for 5 minutes at 4 °C. Carefully aspirate the supernatant.
(Optionally) Wash cells by adding cold PBS. Spin down the cells for 5 minutes at an appropriate centrifugal force and aspirate supernatant completely.
Note: Please make sure that cells do not get damaged during centrifugation.
3. Quick-freeze cell pellet by complete submersion in liquid nitrogen for at least 15 seconds.
4. Ship samples on dry ice.

Suspension cells

1. Transfer cells to a tube. Pellet the cells by centrifuging at an appropriate centrifugal force such as 100–500×g for 5 minutes at 4 °C. Carefully aspirate the supernatant.
(Optionally) Wash cells by adding cold PBS. Spin down the cells for 5 minutes at an appropriate centrifugal force and aspirate supernatant completely.
Note: Please make sure that cells do not get damaged during centrifugation.
2. Quick-freeze cell pellet by complete submersion in liquid nitrogen for at least 15 seconds.
3. Ship samples on dry ice.

3. Quick-frozen tissue samples

Remove fat and other components that do not belong to the sample before quick-freezing the tissue biopsy.

1. Quick-freeze samples immediately in liquid nitrogen and (optional) store them in a freezer at –80 °C. If only a part of the sample has to be processed, crush frozen samples under liquid nitrogen or directly cut samples before quick-freezing.
2. Ship samples on dry ice.
Note: Frozen samples must not thaw.