

Please fill out the order confirmation completely and correctly to avoid errors and delays and fax it to Miltenyi Biotec Inc.  
**Fax no. +1 530 887 5399.**

If you need assistance, call technical support at 800-367-6227.  
 Please send your samples and a copied order confirmation form to:

**Miltenyi Biotec Inc.**  
 2303 Lindbergh Street  
 Auburn, CA 95602



**Microarray Service  
 Order Confirmation**

**PIQOR™ Microarray Service  
 Agilent Whole Genome Microarray Service\***

**1. Which microarray service should be performed ?**

**1.1 PIQOR™ Microarray Service**

**Please specify the microarray:**

- |                                     |                                    |  |
|-------------------------------------|------------------------------------|--|
| <input type="checkbox"/> Cell Death | <input type="checkbox"/> Skin      | <input type="checkbox"/> Toxicology    |
| <input type="checkbox"/> Immunology | <input type="checkbox"/> SkinPatho | <input type="checkbox"/> Ubiquitin-PSI |
| <input type="checkbox"/> Oncology   | <input type="checkbox"/> Stem Cell | <input type="checkbox"/> Custom        |

**Species:**  human  mouse  rat

**Quantity of microarrays:** \_\_\_\_\_

**Please specify the PIQOR™ Service:**

- Standard (without amplification): 50 µg total RNA required; approximately 2x10<sup>6</sup> cells or 40 mg tissue.
- With T7 Plus Amplification: 1 µg total RNA required; approximately 1x10<sup>5</sup> cells or 1 mg tissue.
- With SuperAmp™ Service: 1–100,000 cells or comparable amount of tissue required; includes delivery of SuperAmp Preparation Kit for sample lysis and storage.

**1.2 Agilent Whole Genome Microarray Service**

**Please specify the Agilent Microarray Service:**

- Two-Color, 4x44K format: 1 µg total RNA required; approximately 1x10<sup>5</sup> cells or 1 mg tissue.
- One-Color, 4x44K format: 1 µg total RNA required; approximately 1x10<sup>5</sup> cells or 1 mg tissue.
- With SuperAmp Service: 1–100,000 cells or comparable amount of tissue required; includes delivery of SuperAmp Preparation Kit for sample lysis and storage.

**Species:**  human  mouse  rat  other \_\_\_\_\_

**Quantity of microarrays:** \_\_\_\_\_

**2. Which samples are you sending? Please specify amount**

Total RNA (µg/µL): \_\_\_\_\_  tissue (mg): \_\_\_\_\_  cells (mg): \_\_\_\_\_  blood (mL): \_\_\_\_\_

How many samples are you sending? \_\_\_\_\_

Shipment of samples (except blood):  frozen on dry ice  stabilized in PrepProtect™ Stabilization Buffer

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

**For SuperAmp™ Service only:**  cells sorted using MACS® Technology  cells sorted by flow cytometry  
 microdissected cells  others \_\_\_\_\_

**Note:** Samples for SuperAmp Service have to be lysed and stored using the SuperAmp Preparation Kit.

**3. Should samples be pooled?**

No  Yes Which samples should be pooled? \_\_\_\_\_

\*Miltenyi Biotec is a certified Agilent microarray service provider.

## 4. Hybridization scheme

Please fill out the hybridization scheme to advise which samples have to be labeled with Cy3- or Cy5-CTP.

For **Two-Color** experiments, please fill out the columns for Cy3 and Cy5, both fluorochromes will be hybridized against each other. Normally, controls will be Cy3-CTP-labeled.

For **One-Color** experiments, just fill out the Cy3 column. The absolute signal intensities are provided in the data output files. Furthermore, we offer „virtual ratio“ analysis to allow fold-change comparison to control. Please mark the control sample in the Cy3 column with an asterisk (\*) against which you would like to compare the other One-Color experiments. If no control is chosen, no ratios can be provided.

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

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

## 5. Order confirmation

Hereby I order for the above listed samples the Microarray Service according to the quote no. \_\_\_\_\_

Institution/Company	
Full name	
Department	
Name of principal investigator	
Address	
Zip code	
Town	
Country	
Phone	
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.

Has **Purchase Order** been sent? Please fax Purchase Order to this number: **+1 530 887 5399**. Samples cannot be processed without a signed order document.

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

The yield of total RNA, which can be extracted from cells or tissue, is highly dependant 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 on our website.

When preparing biological samples, e.g. cultured or primary cells, or tissue, work quickly until the sample is either quick-frozen in liquid nitrogen or stabilized in PrepProtect™ Stabilizing Buffer (10 mL, # 130-092-643; 100 mL, # 130-092-642). PrepProtect Buffer is a non-toxic reagent that stabilizes both freshly prepared as well as previously frozen samples since it prevents RNA degradation.

**Note:** Use appropriate storage vessel, e.g. Eppendorf® micro test tubes or BD Falcon® 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 or cool packs for samples stabilized in PrepProtect Buffer are 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 for SuperAmp™ Service (1.), cell (2.), tissue (3.), or whole blood (4.) samples for all Microarray Services without SuperAmp Amplification Service.

### 1. Samples for SuperAmp™ Service

Samples are lysed with the SuperAmp Lysis Buffer and stored according to the guidelines provided in the SuperAmp Preparation Kit. Samples are shipped on dry ice to Miltenyi Biotec. Please follow instructions specified in the data sheet and use the provided SuperAmp Tube Container for shipment.

### 2. Cells

#### 2.1 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.

#### 2.2 Cell pellets stabilized in PrepProtect™ Buffer

1. Resuspend freshly harvested cells in a small amount of PBS, add 5–10 volumes of PrepProtect Buffer, mix by inverting the tube or by shaking a microtiter plate, and spin down shortly to collect sample at the bottom.
2. Fill up tube with PrepProtect Buffer.
3. Ship samples with cool packs. For further information please refer to the PrepProtect Buffer data sheet.

### 3. Tissue

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

#### 3.1 Quick-frozen tissue

1. Quick-freeze samples immediately in liquid nitrogen and (optional) store them in a refrigerator 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 except when stabilized in PrepProtect Stabilizing Buffer (see below).

#### 3.2 Tissue stabilized in PrepProtect Buffer

1. Immediately submerge freshly dissected tissue piece, max. 5 mm in any one dimension, completely in 5–10 volumes of PrepProtect Buffer and invert the tube several times.  
**Note:** Optimally, the tissue floats freely in PrepProtect Buffer.
2. Fill up the tube with PrepProtect Buffer. Incubate tissue overnight at 4–22 °C.
3. Ship samples with cool packs. For further information please refer to the PrepProtect Buffer data sheet.

### 4. Whole blood samples

#### 4.1 Whole blood (stabilized)

1. Collect 2.5 mL fresh blood in PAXgene™ Blood RNA Tubes, available from BD cat. no. 762165. The PAXgene Blood RNA Tubes should only be frozen in a wire rack, not in a styrofoam tray.
2. Freeze the tubes at –20 °C and then transfer to –70 °C.
3. Ship the tubes with stabilized sample on dry ice.

**Note:** RNA purified from reticulocytes contains a lot of alpha and beta globin transcripts.

#### 4.2 Cells sorted with MACS® Technology (quick-frozen or stabilized in PrepProtect Buffer)

1. Separate white blood cells or subpopulations of blood cells out of whole blood with the autoMACS™ Separator. Alternatively, subpopulations of blood cells can be isolated from buffy coats with MACS Technology.
2. 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.  
**Note:** Please make sure that cells do not get damaged during centrifugation.
3. Quick-freeze or stabilize with PrepProtect Buffer. For details regarding transport, please refer to section 2. Cells.



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