

Monocyte (CD14⁺) Total RNA

human

Cell type-specific total RNA for gene cloning and gene expression analysis Order no. 130-093-166

Index

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Reagent and instrument requirements
2. Product quality
 - 2.1 Blood donation
 - 2.2 Cell preparation
 - 2.3 Cell type-specific total RNA preparation and purity
3. Examples
4. References

1. Description

| | |
|-------------------|---|
| Components | 5 µg Monocyte (CD14 ⁺) Total RNA, human (lyophilized). |
| Size | 5 µg total RNA. |
| Source | Healthy human blood donors. |
| Storage | Dissolve cell type-specific total RNA in 50 µL sterile, RNase-free distilled water and prepare aliquots. Store dissolved total RNA and lyophilized total RNA at -70 °C. |

1.1 Background information

Monocytes belong to the classical antigen-presenting cells. Antigen uptake takes place via phagocytosis or receptor-mediated endocytosis. CD14 is expressed strongly on monocytes and macrophages.

Monocyte (CD14⁺) Total RNA has been isolated from highly purified human CD14⁺ monocytes.

1.2 Applications

The RNA is suitable for gene expression profiling, cDNA library generation, RT-PCR analysis, cloning and characterization of monocyte-specific genes.

1.3 Reagent and instrument requirements

- Sterile, RNase-free distilled water.

2. Product quality

2.1 Blood donation

Donors were consenting, healthy, normal adults aged between 18 and 68 years. The donation procedure was performed following the guidelines of the German Medical Association and the regulations of German and EU law. Donors were negative for HIV, hepatitis B and C, and syphilis. Donors undergoing a course of medication were excluded.

2.2 Cell preparation

Peripheral blood mononuclear cells (PBMCs) were prepared from buffy coats, collected from multiple donors, by Ficoll[®] density gradient centrifugation. CD14⁺ cells were isolated from the PBMCs by positive magnetic selection using CD14 MicroBeads, human (# 130-050-201). Purities always exceeded 90% CD14⁺ cells.

2.3 Cell type-specific total RNA preparation and purity

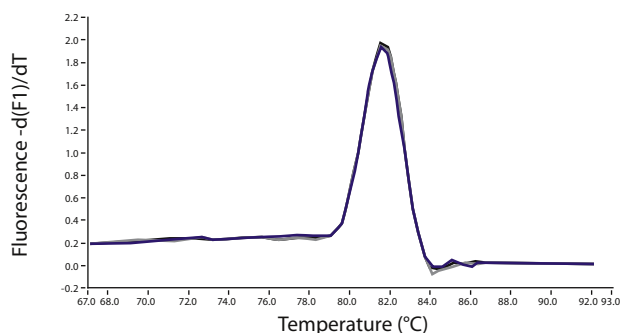
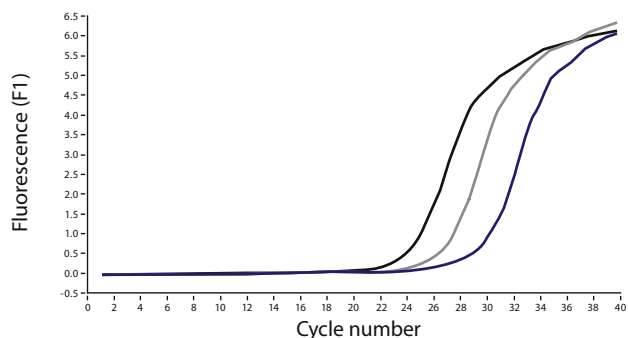
Pooled, purified cells from multiple donors were lysed with RNA lysis buffer and total RNA was extracted by using silica-membrane technology. The RNA was treated with RNase-free DNase I to remove residual contamination with genomic DNA. After treatment DNase was removed and inactivated. RNA purity was determined by capillary electrophoresis with an Agilent Bioanalyzer and consistently showed RNA Integrity Number (RIN) values^{1,2} of over 8.5.

Table 1: Donor, cell, and RNA quality parameters

| Parameter | Value |
|---|-------------------------------------|
| Donor-age distribution | 18–68 years |
| Donor gender | 50% male, 50% female |
| Viral serology of donors | Negative for HIV, HBV HCV, syphilis |
| Donor medication | Negative |
| Cell purity | > 90% CD14 ⁺ |
| RNA Integrity Number (RIN) ^{1,2} | > 8.5 |

3. Examples

The Hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene is a housekeeping gene that is normally expressed at a frequency of 1–10 mRNA copies per cell;³ therefore, it provides a suitable reference for RNA quality. To determine HPRT mRNA content 1 ng, 10 ng, and 100 ng of monocyte (CD14⁺) Total RNA were analyzed by real-time RT-PCR using intron-spanning primers.



4. References

1. Schroeder, A. *et al.* (2006) The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology* 2006, 7: 3
2. Imbeaud, S. *et al.* (2005) Towards standardization of RNA quality assessment using user-independent classifiers of microcapillary electrophoresis traces, *Nucl. Acids Res.* 2005 33: e56.
3. Steen, A. M. *et al.* (1990) Levels of hypoxanthine phosphoribosyltransferase RNA in human cells. *Exp. Cell Res.* 186: 236–244.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

MACS is a registered trademark of Miltenyi Biotec GmbH.

Ficoll-Paque is a registered trademark of GE Healthcare UK Ltd.

© 2007 Miltenyi Biotec GmbH.