MACS molecular

T Helper Cell ($CD4^+$) Total RNA

human

Cell type-specific total RNA for gene cloning and gene expression analysis Order no. 130-093-163

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1. Description

Components	5 µg T Helper Cell ($CD4^+$) 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

$CD4^+$ helper T cells have a number of functions: activation of B cells leading to their differentiation into antibody-secreting plasma cells or memory B cells; control of isotype switching in reponse to released cytokines; and initiation of somatic hypermutation of the antibody variable V-region genes.

T Helper Cell ($CD4^+$) Total RNA has been isolated from highly purified human $CD4^+$ T helper cells.

1.2 Applications

The RNA is suitable for gene expression profiling, cDNA library generation, RT-PCR analysis, cloning, and characterization of T helper cell-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 (PMBCs) were prepared from buffy coats, collected from multiple donors, by Ficoll™ density gradient centrifugation. $CD4^+$ T helper cells were isolated from the PBMCs by positive magnetic selection using CD4 MicroBeads, human (# 130-045-101). Purities always exceeded 90% $CD4^+$ T 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% $CD4^+$
RNA Integrity Number (RIN)[1,2]	> 8.5

140-002-064.02

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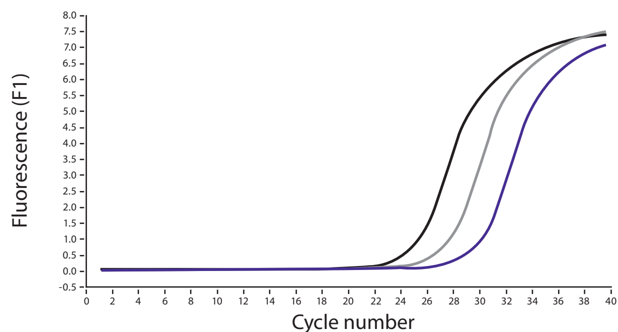
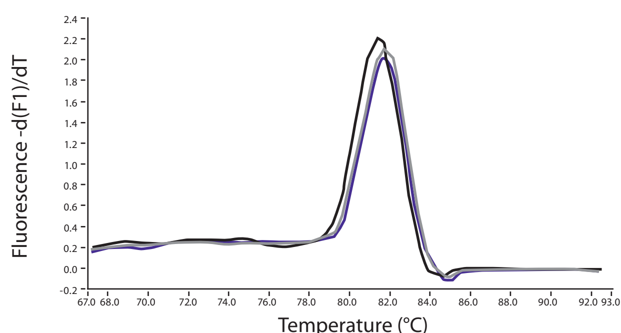
3. Examples

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 T Helper Cell ($CD4^+$) 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.

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140-002-064.02

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