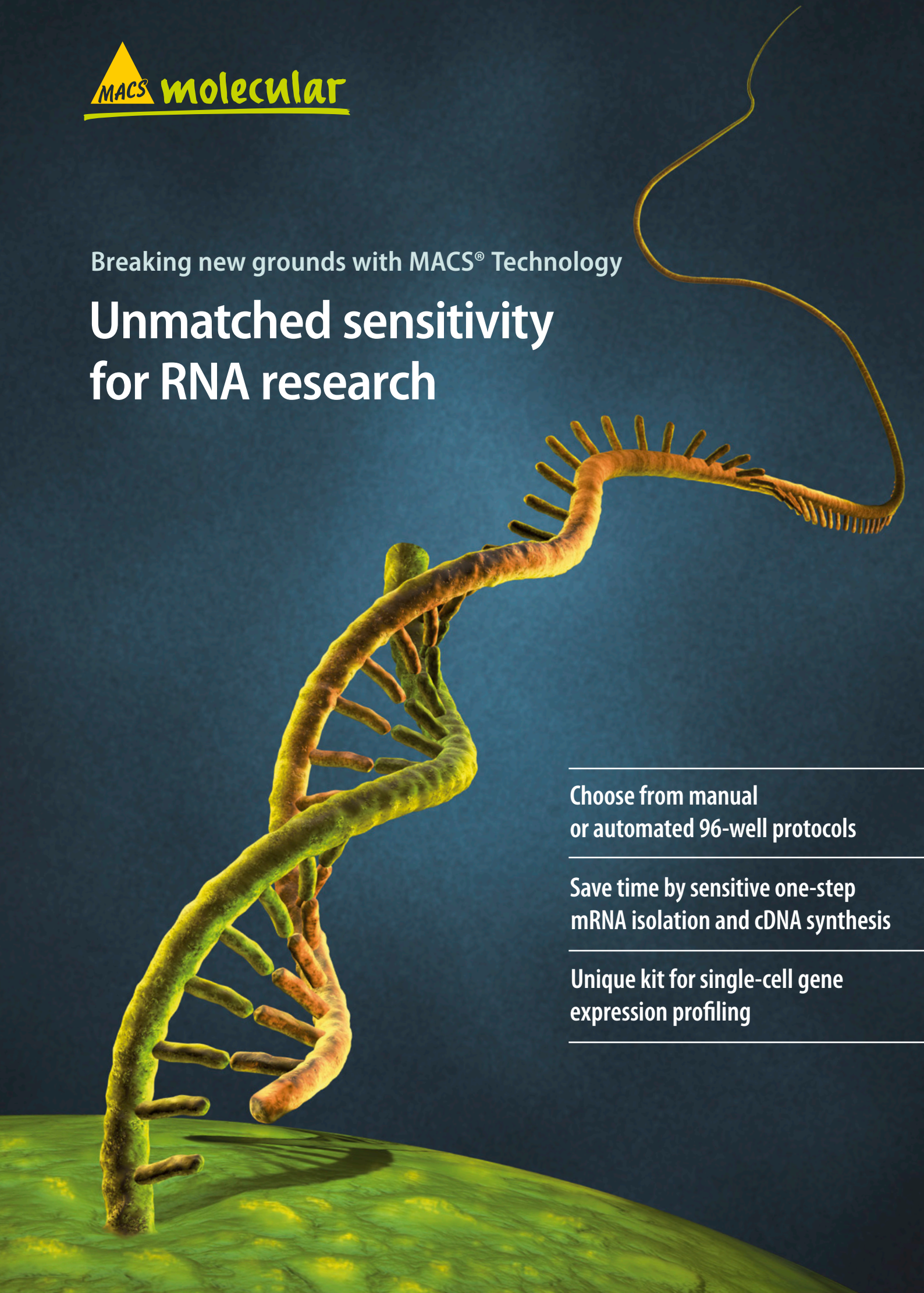
MACS molecular

Breaking new grounds with MACS® Technology

Unmatched sensitivity for RNA research



Choose from manual or automated 96-well protocols

Save time by sensitive one-step mRNA isolation and cDNA synthesis

Unique kit for single-cell gene expression profiling

MACS® Technology by Miltenyi Biotec

Comprehensive solutions from cell to molecular analysis

Since its introduction in 1989, MACS® Technology has become the gold standard for cell separation. Nowadays, Miltenyi Biotec stands for more than cell separation, offering over 1000 innovative research products for biomedical research and life sciences. The MACS Product portfolio includes instruments and reagents for sample preparation, cell separation, cell analysis, cell culture, and molecular biology. Over the last 20 years, researchers have published more than 14,500 papers with our products. Miltenyi Biotec has a strong commitment to constantly develop new products for current and future research.

Sample preparation

- Fast and gentle sample preparation from any tissue
- Get viable single cells for cell separation, analysis, or cultivation
- Get cell homogenates for molecular analysis

Cell separation

- MACS® Technology, the gold standard in cell separation
- Manual or automatic separation of virtually any cell type
- Standardization with automated cell separation

Cell analysis

- Titrated high-quality antibodies and brilliant fluorochromes
- Easy-to-use best in class flow cytometers

Cell culture

- Serum-free medium and supplement for long-term viability
- Premium, GMP, and research grade cytokines

Molecular analysis

- Fast isolation of functional mitochondria
- Sensitive protein isolation even from small samples
- Efficient mRNA isolation and amplification from small samples
- Genomic Services for gene and microRNA expression profiling and array CGH

Imaging

- Contrast agents optimized for small animal imaging
- MRI, optical imaging, CT and ultrasound

MACSmolecular products

MACS® Technology for molecular applications

Products for highly sensitive RNA analysis

In many research fields, gene expression analysis is a standard approach to gain a better understanding of particular biological processes. Researchers either focus on single genes via PCR analysis, or they monitor whole genomes using microarray analysis.

Accurate gene expression analyses depend on mRNA isolation methods that circumvent common pitfalls, such as DNA contaminations or degradation of RNA during isolation. Significant amounts of mRNA are often lost during precipitation and washing steps. In particular, when sample material is limited to a few cells a reliable technology for efficient mRNA isolation and cDNA synthesis is required.

MACS® Technology takes care of this necessity: Outstanding RNA recovery during isolation is achieved by utilizing the superparamagnetic MicroBeads. These 50-nm particles instantly bind to their target poly-A tail and allow mRNA isolation even from a single cell. The innovative in-column cDNA synthesis reduces loss of material and further increases the sensitivity and reliability of your RNA analysis.

The new µMACS™ SuperAmp™ Kit allows whole genome analysis from just one cell up to 10,000 cells. Thus, Miltenyi Biotec products for RNA research are ideally suited for assaying small samples.

Contents

4 How MACS® Technology works
For one-step mRNA isolation and cDNA synthesis

6 mRNA isolation and cDNA synthesis
Manual sample preparation for PCR analysis

8 96-well mRNA isolation and cDNA synthesis
Automated sample preparation for PCR analysis

10 Single-cell gene expression profiling
SuperAmp™ Technology for unmatched sensitivity in microarray analysis

12 Microarray Services for RNA research
Expert consultation right from the start

13 More products for RNA research
Sample preparation and stabilization, reference RNA, and *in situ* hybridization

14 Product overview
Place your order by fax, phone, or online

15 References
Selected from more than 14,500 papers using products from Miltenyi Biotec

How MACS® Technology works

For one-step mRNA isolation and cDNA synthesis

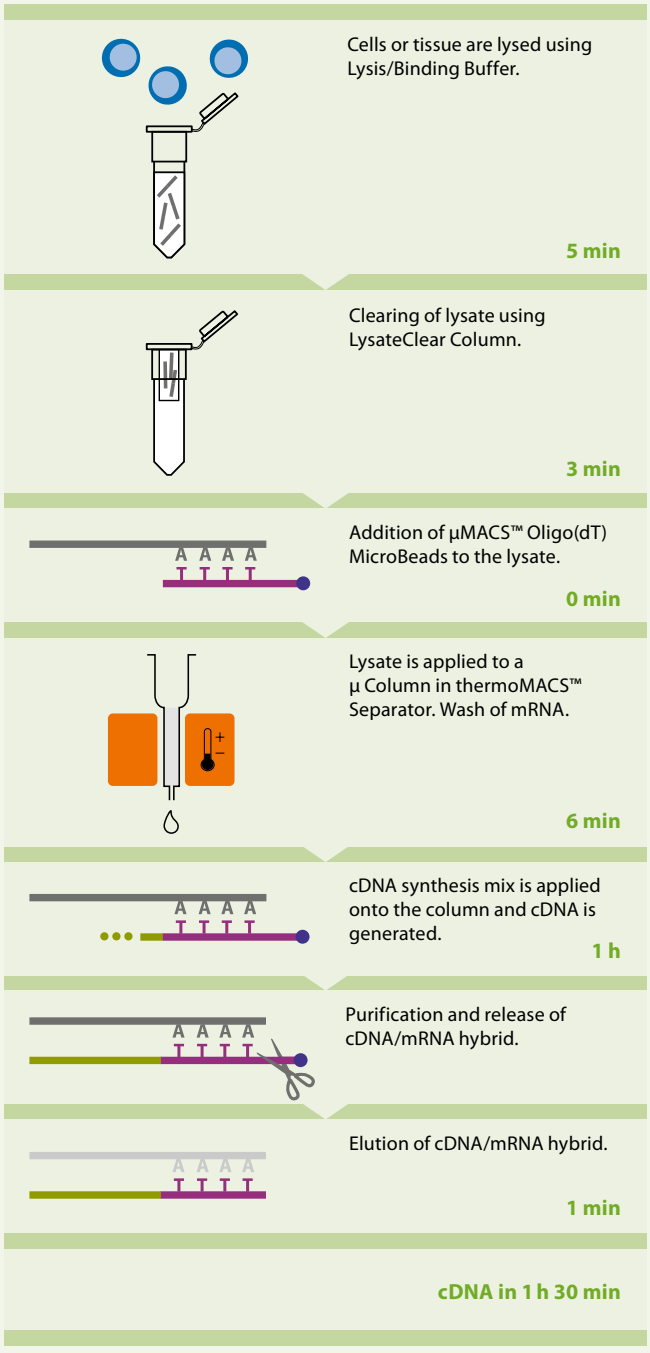


Fig. 1: Principle of MACS Technology for mRNA isolation and cDNA synthesis

The operating principle

Superparamagnetic µMACS™ Oligo(dT) MicroBeads are applied to the cell lysate and instantly bind to their target mRNA. Subsequently, the magnetically labeled mRNA is isolated and purified in a MACS Column placed in the magnetic field of a MACS Separator. Reverse transcription takes place in the same column. After thorough washing, pure cDNA is eluted (for details, please refer to figure 1).

How you benefit from MACS® Technology

Benefit from MACS Technology for one-step mRNA isolation and cDNA synthesis:

Outstanding sensitivity

A few cells are enough.

High purity

No contamination with genomic DNA.

High recovery

Preserve sample material due to omission of centrifugation steps or buffer removal.

Reproducibility and reliability

One-step cDNA synthesis procedure can be easily automated.

The components

1. µMACS™ Oligo(dT) MicroBeads

- Superparamagnetic — only magnetized in a magnetic field
- Small in size — only 50 nm in diameter
- Non-sedimenting with extremely high reaction kinetics — instantly bind to target mRNA

Benefit: Outstanding sensitivity — mRNA can be isolated from as few as a single cell.

Fig. 2: µMACS Oligo(dT) MicroBeads

2. MACS® Column

- Packed with steel spheres that enhance the magnetic field — essential to retain nanometer-sized µMACS™ MicroBeads bound to target mRNA
- Buffers run by gravity flow, thus, no need for centrifugation or buffer removal steps. Loss of target is prevented.
- Thorough rinsing procedure

Benefit: High recovery and purity.

Fig. 3: MACS Column

3. MACS Separators

MACS Separators are permanent magnets. The heatable thermoMACS™ Separator was developed especially for in-column enzymatic reactions such as cDNA synthesis of isolated mRNA. The one-step approach reduces loss of material by avoiding tube-to-tube transfer.

Benefit: High recovery

Automated 96-well processing

The procedure can easily be scaled-up to a 96-well format utilizing the MultiMACS™ M96thermo Separator. A fully automated approach is achieved by integrating this benchtop instrument into a robotic pipetting system.

Benefit: High reproducibility and reliability.

Fig. 4: MultiMACS M96thermo Separator

Fig. 5: thermoMACS Separator

mRNA isolation and cDNA synthesis

Manual sample preparation for PCR analysis

Fig. 1: µMACS mRNA Isolation Kit

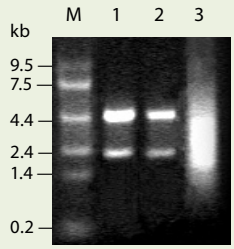


Fig. 2: mRNA was isolated from total RNA of a hybridoma cell line
Agarose gel lane 1 shows total RNA (Trizol™, Invitrogen), lane 2 contains flow-through wash buffer, and lane 3 reflects mRNA isolated by MACS Technology; M, marker.

Fig. 3: thermoMACS™ Separator

Outstanding sensitivity — a few cells are enough

The **µMACS™ mRNA Isolation Kits** yield full-length, intact mRNA from up to 10^7 cells (fig. 2). Even when starting with small cell numbers, this magnetic bead–based technology ensures reliable results.

Due to the fast reaction kinetics of the extremely small (50 nm) MicroBeads, mRNA molecules are instantly bound and high-purity mRNA can be successfully isolated from very small samples.

High purity

MACS® Technology enables a thorough in-column washing procedure. Premium mRNA can be isolated without any genomic DNA contamination (fig. 5B).

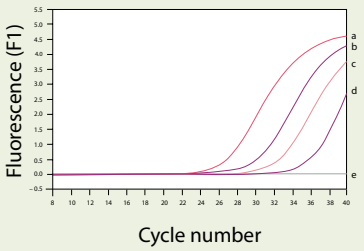
Direct isolation of mRNA from various samples

High-quality mRNA can be isolated directly from various biological materials. It works like a charm for all cell lines, for primary cells and also for tissue.

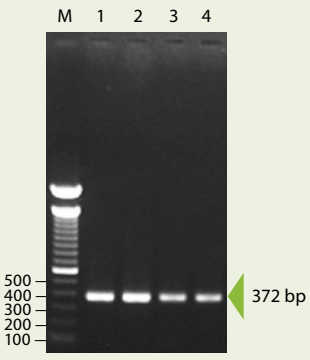
mRNA in 15 minutes

mRNA isolation directly from cell, tissue, or blood samples takes only 15 minutes. Within a further 75 minutes the cDNA can be easily synthesized and purified in the same column that was used for mRNA isolation.

Fig. 4: The thermoMACS™ Separator can be heated to 37 °C or 42 °C



A) mRNA was isolated and reverse transcribed into cDNA by µMACS One-step cDNA Kit (a: 500 cells; b: 5 cells) or by conventional tube methods (c: 500 cells; d: 5 cells). e: non-template control. 10% of each cDNA was analyzed by quantitative PCR using intron-spanning primers.



B) µMACS One-step cDNA synthesis (lanes 1 and 2: 500 Jurkat cells; lanes 3 and 4: 5 Jurkat cells, M: 100 bp ladder). 50% of each cDNA was used in a standard PCR reaction. A 372 bp fragment of GAPDH was amplified with intron-spanning GAPDH primers (genomic DNA would give a 476 bp fragment) and analyzed by agarose gel electrophoresis.

Fig. 5: Sensitive generation of PCR template using µMACS Technology
PCR analysis of GAPDH fragment using mRNA isolated from 500 or 5 Jurkat cells by quantitative PCR (A) and agarose gel electrophoresis (B).

Unique one-step mRNA isolation and cDNA synthesis

Instead of eluting the purified mRNA from the column (refer to page 4), a first-strand cDNA synthesis can be performed directly in the column employing the µMACS™ One-step cDNA Kit and the thermoMACS™ Separator. The latter is a permanent magnet that can be heated to 37 °C or 42 °C.

Five cells are enough for PCR analysis

The revolutionary in-column cDNA synthesis (described on page 4) significantly reduces loss of mRNA and cDNA. Magnetically bound mRNA is directly reverse transcribed in the column using a highly efficient reverse transcriptase. After first-strand synthesis, unwanted components are washed away while the cDNA is retained in the column.

This unique technology preserves sample material at each step, as mRNA isolation, cDNA synthesis and purification are all performed within the same column. Even when starting with small samples, such as five cells, reliable cDNA synthesis can be achieved (fig. 5).

> **"It is highly reliable … and yields a large amount of pure product."**
>
> **"In contrast to the other techniques, genomic DNA amplification was detected in none of the samples that had been processed by magnetic bead isolation."**
>
> Mack *et al.* (2007) Cytometry A. 71: 404 – 409.

96-well mRNA isolation and cDNA synthesis

Automated sample preparation for PCR analysis

Fig. 1: MultiMACS M96thermo Separator for 96-well mRNA isolation and cDNA synthesis

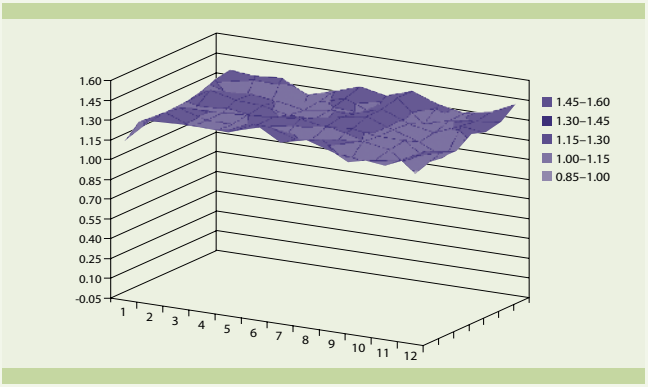


Fig. 2: Quantification of 96 mRNA samples
Using the MultiMACS M96 Separator combined with the MultiMACS mRNA Isolation Kit, 100 µg of mouse liver total RNA was purified and quantified. Average yield: 1.3 µg mRNA; coefficient of variation of 96 samples: 5.2%.

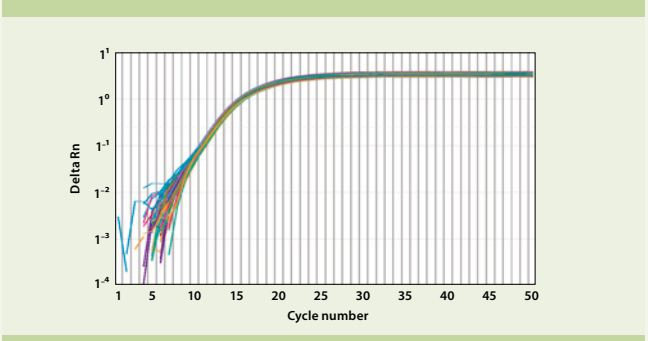


Fig. 3: Quantitative RT-PCR of 96 samples using the MultiMACS System
100 µg total RNA was purified from mouse liver and subsequently reverse transcribed by using the MultiMACS cDNA Synthesis Kit and the MultiMACS Separator. After reverse transcription, quantitative amplification of the housekeeping gene GAPDH was measured by real-time PCR. Mean cycle threshold: 12.0; coefficient of variation: 2.0 %.

Automated 96-well mRNA isolation

For multi-specimen PCR screening the proven MACS® Technology for mRNA isolation (refer to page 6) can be scaled up to a 96-well format.

The **MultiMACS™ mRNA Isolation Kits** are used in combination with the MultiMACS M96 Separator. This benchtop instrument contains a 96-well magnet and allows the simultaneous mRNA purification of multiple samples in less than 45 minutes directly from cells or tissues.

The **MultiMACS M96 Separator** can be operated manually at the bench or automatically by integrating it with a robotic pipetting system (figs. 4 and 5).

Fig. 4: Manual sample processing

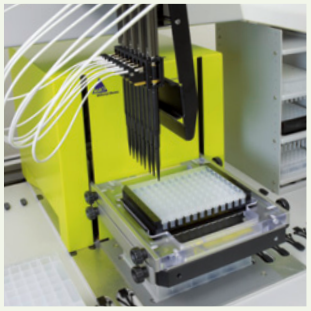


Fig. 5: Automatic sample handling

High reliability and reproducibility

Using the MultiMACS mRNA Isolation Kits and the MultiMACS cDNA Synthesis Kits (refer to next page), the variation in yield and quality of mRNA and cDNA as well as real-time PCR thresholds are very low (figs. 2 and 3).

"We usually process several dozen samples. Now, with the MultiMACS M96thermo Separator we can do parallel cDNA synthesis from up to 96 samples and save a lot of time and costs."

Prof. C. Wolfrum, ETH Zurich, Switzerland

Automated 96-well cDNA synthesis

The MultiMACS™ M96 Separator is also available with a heatable magnet and is then termed the **MultiMACS M96thermo Separator** (fig. 1). Thus, the one-step mRNA isolation and cDNA synthesis procedure can be scaled-up to fully-automated, parallel processing of 96 samples.

Use the **MultiMACS cDNA Synthesis Kits** in combination with the MultiMACS M96thermo Separator to automate your mRNA isolation and cDNA synthesis protocol and benefit from the advantages of MACS® Technology:

- One step — mRNA isolation, cDNA synthesis and purification are performed in one column
- Extremely sensitive — five cells are enough for PCR analysis
- High purity — no contamination of genomic DNA
- High reliability and reproducibility
- No cross-contamination

No cross-contamination due to contact-free pipetting

A hallmark of MACS Technology is the gravity-driven column flow. This not only avoids centrifugation steps but also enables contact-free pipetting (fig. 6) as there is no need to remove buffers after washing steps. Thus, the risk of cross-contamination is prevented (fig. 7).

> **"The sensitivity of this in-column cDNA technology is truly benchmark, and I don't know any other technology that allows processing of up to 96 samples resulting in such high-quality cDNA — even without any genomic DNA contamination!"**
>
> Prof. C. Wolfrum, ETH Zurich, Switzerland

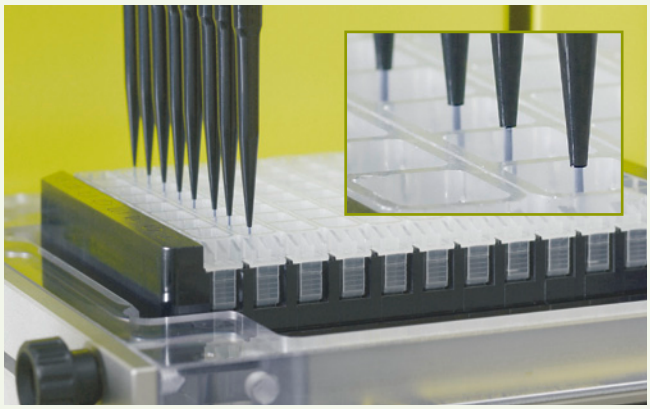


Fig. 6: Contact-free pipetting for reduced risk of cross-contamination

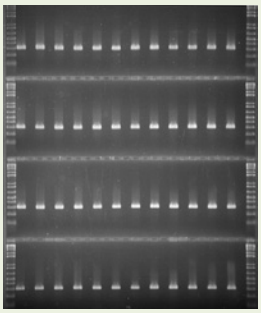


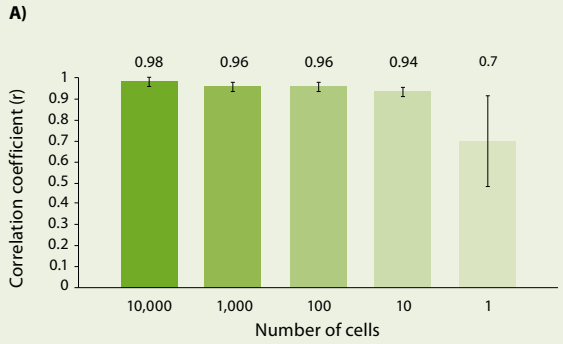
Fig. 7: Analysis of cDNA synthesized with the MultiMACS System
mRNA extracted from 15 mg mouse liver was purified using the MultiMACS Kit and Separator. Liver samples and controls (lysis/binding buffer) were loaded into MultiMACS Sample Plate wells in an alternate fashion, i.e., sample–control–sample–control, etc. RT-PCR was subsequently performed on the contents of each plate well with GAPDH primers (35 cycles). 1 µL of each cDNA product/control was resolved by agarose gel electrophoresis (2 %). It is evident that no cross-contamination occurred between control and liver samples using the MultiMACS System.

Single-cell gene expression profiling

SuperAmp™ Technology for unmatched sensitivity in microarray analysis

µMACS One-step cDNA Labeling Kit	µMACS One-step T7 Template Kit	µMACS SuperAmp Kit
Up to 10^7 cells	1×10^6 – 5×10^4 cells	10^4 – a single cell
Up to 30 mg human and animal tissue (100 mg plant tissue)	6 mg – 500 µg tissue	
200 µg – 5 µg total RNA	10 µg – 250 ng total RNA	100 ng – 10 pg total RNA
No amplification	1000-fold amplification	Millionfold amplification

Table 1: Amplification efficiencies of µMACS Products



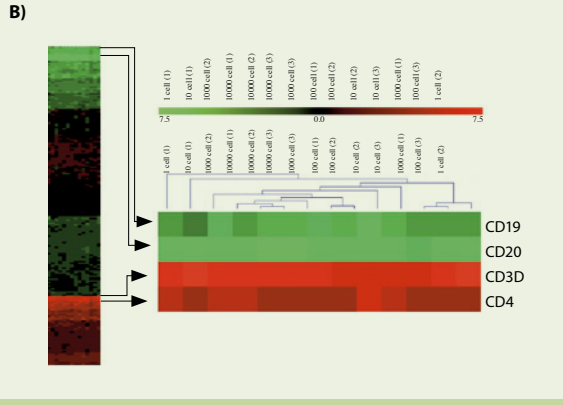


Fig. 1: Linearity analysis of the SuperAmp Protocol
cDNAs were amplified from 1, 10, 100, 1,000 and 10,000 Jurkat cells, and from 1000 Raji cells using the SuperAmp System. To evaluate linearity each amplified Jurkat cDNA product was labeled and compared to the target Raji cDNA product in two-color PIQOR™ Immunology Microarray experiments. (A) shows the calculated pearson correlation coefficient (r) of independent repeats for each Jurkat cell batch. (B) shows hierarchical clustering analysis: no obvious grouping of repeats or cell numbers is evident.

The optimal solution for all sample sizes — even for single cells

Miltenyi Biotec's MicroBead-based isolation procedure of high-purity mRNA is ideally suited for microarray analysis. Kits are available for all samples sizes (table 1) from 10^7 cells or equivalent amounts of tissue or total RNA to only a single cell when using the µMACS™ SuperAmp™ Technology.

µMACS™ SuperAmp™ Kit — a single cell is enough!

Based on the well-established MACS® Technology the µMACS SuperAmp System allows highly sensitive mRNA isolation, cDNA synthesis, and a millionfold amplification of the mRNA-derived cDNA by global PCR. The principle of the µMACS SuperAmp Process is illustrated in figure 2.

The unique µMACS SuperAmp Protocol combines several features

- Unmatched sensitivity
- High reproducibility

  PCR bias is avoided by the use of uniform primers and uniform annealing conditions for all transcripts. In addition, the consistent length of generated cDNA fragments avoids PCR bias. This process preserves the relative transcript abundance in the sample with virtually no non-specific amplification product.
- Much faster and more reliable than two rounds of T7 amplification
- Easy to use
- Robust and reliable system

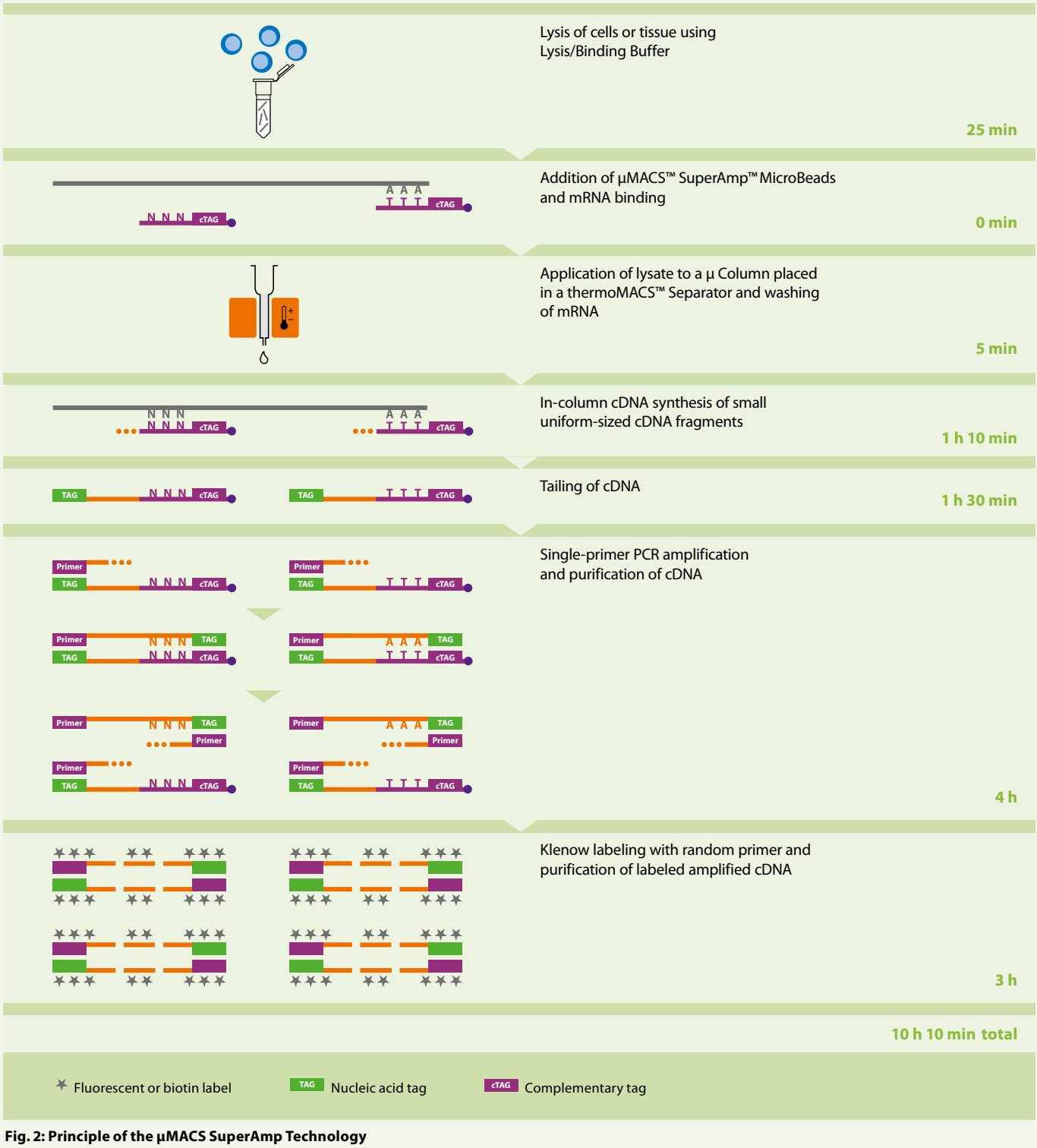


Fig. 2: Principle of the µMACS SuperAmp Technology
µMACS SuperAmp Technology enables gene expression profiling from even the tiniest amounts of sample material.

Microarray Services for RNA research

Expert consultation right from the start

Send sample — receive results

Technical support for experimental design and microarray selection

- microRNA miRXplore™ Microarrays
- Agilent Whole Genome Microarrays

RNA extraction and quality control

Optional:

- Amplification and quality control
- SuperAmp Service*: 1 – 10,000 cells

Synthesis and purification of fluorescently labeled probes

Microarray hybridization

Image capture and analysis of primary data

Optional: **Bioinformatics Services****

- Pre-processing
- Ratio Building
- Cluster
- Discriminatory Genes
- Functional Grouping
- Pathway Analysis

Results and report

- Data on CD-ROM

* microRNAs cannot be amplified with the SuperAmp Service.
** Please inquire for microRNA Bioinformatics Services.

Ten years of microarray experience

Miltenyi Biotec has ten years of microarray experience and offers a huge variety of genomics services. Since 2003, we are also an officially certified Agilent Service provider.

There is no need to establish microarray technology in your own laboratory — simply send cells, tissue, or blood samples to our Microarray Services Department and receive reliable results and detailed documentation in return.

Flexible expression profiling services

Miltenyi Biotec provides a wide range of cost-effective microarray services:

- microRNA expression analysis based on miRXplore™ Microarrays
- Agilent whole genome expression analysis
- Agilent array-CGH

SuperAmp™ Service for one-cell microarray experiments

The SuperAmp Service is an extension of the Microarray Services and allows successful gene expression profiling from 1 – 10,000 cells.

Find detailed information on our extensive Genomic Services at **www.miltenyibiotec.com/genomic-services**

More products for RNA research

Sample preparation and stabilization, reference RNA, and *in situ* hybridization

gentleMACS™ Dissociators

The isolation of subcellular material such as total RNA or mRNA from tissues or cells requires fast and thorough homogenization of the starting material. The gentleMACS™ Octo Dissociator and gentleMACS™ Dissociator (fig. 1) provide optimized homogenization programs that meet these requirements. For further information, refer to **www.gentlemacs.com**

Fig. 1: gentleMACS™ Octo Dissociator and gentleMACS Dissociator

PrepProtect™ Stabilization Buffer

Simply by adding the non-toxic PrepProtect™ Solution, RNA, DNA, or protein is stabilized in cell or tissue samples and is available for reliable downstream applications.

PrepProtect Stabilization Buffer prevents RNA degradation whenever quick-freezing or direct analysis after sample collection is impossible, for example, when samples are shipped without dry ice or frozen tissue is weighed and dissected.

Cell type–specific total RNA

Highly pure total RNA from distinct hematopoietic cell populations such as hematopoietic progenitor cells ($CD34^+$) or regulatory T cells ($CD4^+$ $CD25^+$) is ready to use in gene expression profiling experiments, gene cloning, or as a reference for further analyses.

EasyProbe Transcription Templates for *in situ* hybridization or Northern blots

Sequence-verified EasyProbe Transcription Templates are linear, double-stranded DNA templates for *in vitro* transcription to generate gene-specific RNA probes for *in situ* hybridization or Northern blot analysis.

The trancription templates are specific for human skin-related and mouse neural mRNAs. Solubilized after delivery, the template can be labeled with either fluorophores, digoxygenine, biotin, or radioactive isotopes.

For detailed information on cell type–specific total RNAs and EasyProbe Transcription Templates, refer to **www.miltenyibiotec.com**

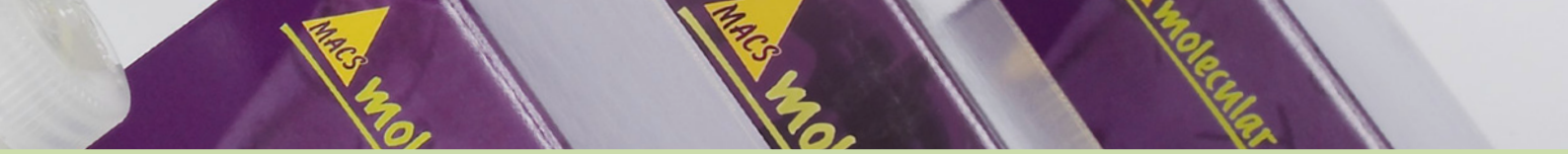
Product overview

Place your order by fax, phone, or online!

Products	Capacity	Order no.
mRNA isolation and cDNA synthesis		
µMACS mRNA Starting Kit	20 reactions	130-075-202
µMACS mRNA Isolation Kit, Small Scale	10 reactions	130-090-276
µMACS mRNA Isolation Kit, Small Scale	20 reactions	130-075-201
µMACS mRNA Isolation Kit, Large Scale	4 reactions	130-090-277
µMACS mRNA Isolation Kit, Large Scale	8 reactions	130-075-101
µMACS mRNA Isolation Kit, For Total RNA	8 reactions	130-075-102
µMACS One-step cDNA Starting Kit	20 reactions	130-091-989
µMACS One-step cDNA Kit	20 reactions	130-091-902
96-well mRNA isolation and cDNA synthesis		
MultiMACS mRNA Isolation Kit (12×8)	96 reactions	130-092-520
MultiMACS mRNA Isolation Kit (4×96)	384 reactions	130-092-519
MultiMACS cDNA Synthesis Kit (12×8)	96 reactions	130-094-410
MultiMACS cDNA Synthesis Kit (4×96)	384 reactions	130-094-408
cDNA labeling and amplification		
µMACS One-step cDNA Labeling Starting Kit	20 reactions	130-092-521
µMACS One-step cDNA Labeling Kit	20 reactions	130-092-443
µMACS One-step T7 Template Starting Kit	20 reactions	130-092-943
µMACS One-step T7 Template Kit	20 reactions	130-092-866
µMACS SuperAmp Starting Kit	10 reactions	130-093-251
µMACS SuperAmp Kit	10 reactions	130-093-242
RNA stabilization buffer		
PrepProtect Stabilization Buffer	10 mL	130-092-643
PrepProtect Stabilization Buffer	100 mL	130-092-642

Products	Order no.
Equipment for 96-well approaches	
MultiMACS 96 Separator	130-091-937
MultiMACS M96thermo Separator	130-094-534
Multi-8 Columns, molecular (12×8)	130-092-444
Multi-96 Columns, molecular (4×96)	130-092-445
Multi-8 Filters	130-092-546
Multi-8 Filters and Frames	130-092-548
Multi-96 Filters	130-092-547
Deep Well Block, 2.5 mL	130-092-549

Products	Order no.
Equipment	
µMACS Separation Unit	130-042-602
thermoMACS Separation Unit	130-091-136
MiniMACS Separation Unit	130-042-102
OctoMACS Separation Unit	130-042-109
MACS MultiStand	130-042-303
µ Columns (20 reactions)	130-042-701
M Columns (10 reactions)	130-042-801



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Selected from more than 14,500 papers using products from Miltenyi Biotec

Read for yourself how MACS® Technology performed in a direct comparison of methods for PCR template generation:

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µMACS™ One-step cDNA Technology

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µMACS™ SuperAmp™ Technology

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PrepProtect™ Stabilization Buffer

Mack *et al.* (2007) Cytometry A. 71: 404–409.

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