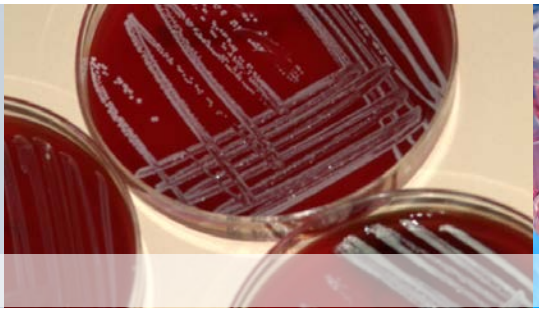
MACHEREY-NAGEL

# NucleoMag® RNA

Automated purification of highly pure RNA from tissue and cells on the platform KingFisher® Flex

TaKaRa
Available from and supported by Takara Bio USA, Inc.


## Introduction

The developmental progress of genome wide transcriptome profiling technologies, such as RNA-Seq, microarray analysis or other methods, enable high sophisticated gene expression studies. Large scale transcriptomic profiling combined with high throughput RNA sequencing helps to e.g., identify unique immune gene expression patterns or cross-cancer gene signatures. The analysis of such expression profiles or genetic biomarkers is critical for developing advanced strategies for infection control or may lead to significant insights into tumorigenesis and metastasis. To generate a robust and reliable transcriptomic profile the isolated RNA needs to be purified from e.g., proteins and contaminants capable to inhibit downstream applications. Moreover the RNA should be of high integrity, free of nucleases and gDNA.

To meet these requirements, MACHEREY-NAGEL developed the NucleoMag® RNA kit, allowing the rapid and reliable purification of highly pure RNA from various tissue samples and cells. The scalable kit was developed for high throughput processing in a 96-well format. The optimized protocol allows the processing of 96 samples within 60 minutes including a rDNase digestion step performed on the KingFisher® Flex platform.

## Product at a glance

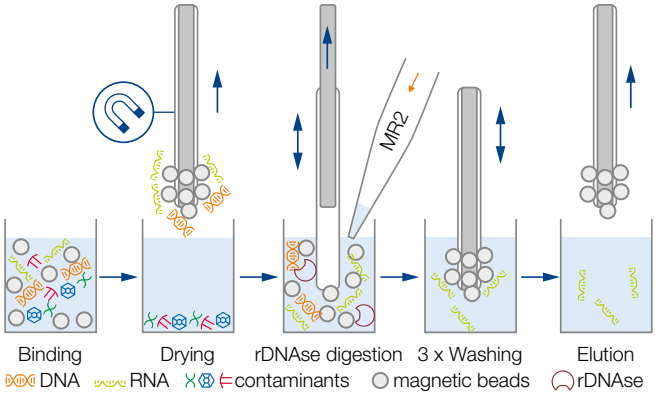
| NucleoMag® RNA | |
|---|---|
| Technology | Magnetic bead technology |
| Sample material | 2 x $10^6$ cells or < 20 mg human / animal tissue |
| Preparation time | Approx. 60 min on KingFisher® Flex for 96 samples including rDNase digestion |
| Typical yield | < 30 µg |
| Elution volume | 50–100 µL |

| King Fisher® Flex | |
|---|---|
| Sample volume | 20–5000 µL |
| Capacity | 24 / 96 samples (8 plates per deck) |
| Heating / cooling | 4–96 °C |
| Size / weight | 60 x 38 x 68 cm / 28 kg |

## Material and methods

Homogenize and lyse up to 20 mg per tissue sample or 2 x $10^6$ cells using the Lysis Buffer MR1 and TCEP. After centrifugation the cleared lysate is transferred to a Square-well Block for further processing.

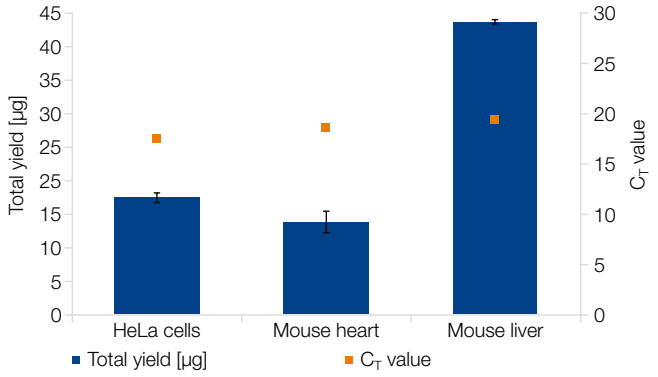
Subsequent DNA isolation is performed on the automation platform KingFisher® Flex. The isolation principle is based on reversible adsorption of nucleic acids to paramagnetic NucleoMag® B-Beads under appropriate buffer conditions.




### Workflow on automation platform

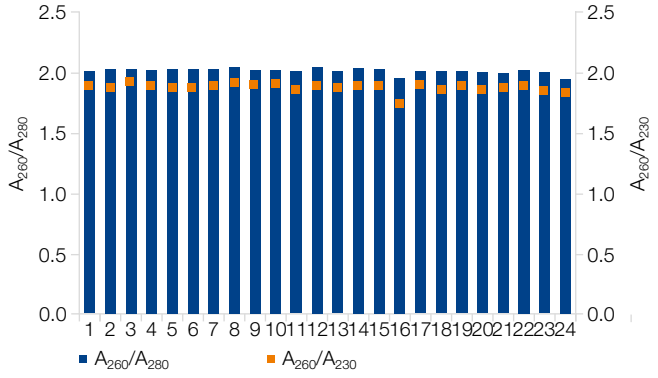
After binding of nucleic acids to the NucleoMag® B-Beads, DNA is digested in a rDNAse reaction mixture and rebinding of RNA to the NucleoMag® B-Beads is achieved by the manual provision of Binding Buffer MR2. After magnetic separation, the paramagnetic beads are washed three times to remove further contaminants and salts using two different Wash Buffers MR3 and MR4. Pure genomic DNA is finally eluted under low ionic strength conditions in the slightly alkaline Elution Buffer MR5.

## Application data



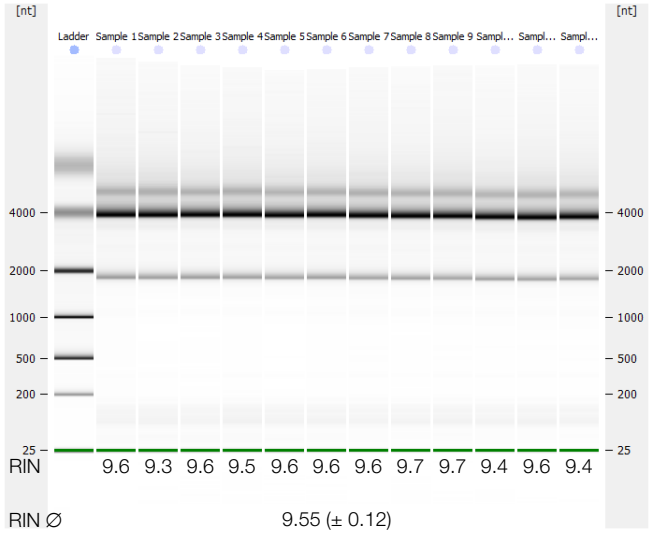

### Isolation of RNA from human cells and animal tissue

Total RNA was isolated from 1 x $10^6$ HeLa cells and different tissue samples stored in RNAlater1 solution using the NucleoMag® RNA kit on a KingFisher® Flex platform. The total yield was determined by UV spectrometry (dark blue bars). A subsequent qRT-PCR analysis (orange squares) was performed with a Taqman® Probe for a 130 bp Actin amplicon using the SensiFast™ Probe Lo-ROX One step kit from Bioline on an Applied Biosystems® 7500 Real-Time PCR System




### Purity of total RNA from human cells

Total RNA was isolated from 1 x $10^6$ HeLa cells (n =24) using the NucleoMag® RNA kit on a KingFisher® Flex platform. The purity was determined by UV spectrometry resulting into an average $A_{260}/A_{280}$ value of 2.01 ± 0.1 (dark blue bars) and into an average. $A_{260}/A_{230}$ value of 1.86 ± 0.1




### Quality of isolated RNA from human cells

After total RNA isolated from twelve individual 1 x $10^6$ HeLa cell samples, the total RNA integrity was determined. RNA was isolated using the NucleoMag® RNA kit on a KingFisher® Flex platform. The quality of the isolated RNA was determined by using the Bioanalyzer® 2100 and the total RNA 6000 Nano kit. The results demonstrate the reliable detection of clear bands for each samples and RIN values constantly above 9 with a mean of 9.55 (Standard deviation of 0.12).

## Automate your RNA extraction from cells and tissue

MACHEREY-NAGEL delivers a ready to go solution for your high throughput RNA extraction from cells and tissue samples. We adapted the NucleoMag® RNA procedure to isolate RNA on instruments of the KingFisher® series to speed up your nucleic acid purification workflow.

- Reliable performance and excellent yields from various sample material
- Speed up your RNA extraction by processing of 96 samples in less than 60 minutes (excluding lysis)

## Ordering information

| Product | Specifications | Preps | REF |
|---|---|---|---|
| NucleoMag® RNA | NucleoMag® B-Beads, buffers, TCEP, RNase-free rDNase | 1 x 96<br>4 x 96 | 744350.1<br>744350.4 |
| KingFisher® Accessory Kit B* | Deep-well Plates, Deep-well Tip Combs, and Elution Plates for 4 x 96 NucleoMag® RNA preps using the KingFisher® Flex platform | 4 x 96 | 744951 |

*For use on KingFisher® Flex