Automated Total RNA Isolation from Cells and Tissue on the MICROLAB® STAR

The isolation of total RNA is automated on the MICROLAB® STAR pipetting robot based on the NucleoSpin® technology from MACHEREY-NAGEL. The isolation of RNA from either cells or tissue can be done with the same method. The throughput of this solution is 96 samples in 60 minutes. The number and type of processed samples are flexible and can be defined by a user input.

Authors

Ms Sylviane Metairon and Mr Frédéric Raymond from Nestlé Research Center, Lausanne/Switzerland, co-operated on the development of the method and co-authored this application note.

Equipment and Materials

Equipment

- MICROLAB® STAR, 8 channels, with built-in robotic plate-handler (iSWAP), manual load
- MICROLAB® BVS Basic Vacuum System incl. ME 4C Vario Membrane Pump and CVC 2000 Controller (Vacuubrand GmbH, Wertheim, Germany)
- All required carriers and the complete method

Reagents

- NucleoSpin® 96 RNA Kit (from MACHEREY-NAGEL GmbH, Düren, Germany)

Protocol

Deck Layout

The deck is manually loaded with carriers containing tips, reagents, filter plates and Eppendorf tubes or micro plates with the samples. The MICROLAB® BVS (Basic Vacuum System) is mounted on a carrier that is fixed to the deck. The plate movements and the loading and unloading of the vacuum box during the process are performed by the iSWAP robotic plate-handler (Figure 1).

Application Software

The validated method was developed with MICROLAB® Vector software. It includes the method itself, labware definitions and liquid classes.

Method

The lysed and prefiltered samples are placed in Eppendorf tubes or microtiter plates on the deck. After the addition of an equal volume of binding buffer and an efficient mixing by aspiration and dispensing steps, the samples are transferred to the NucleoSpin® RNA Binding Plate. During the following filtration step in a vacuum created by the MICROLAB® BVS, the RNA binds to the silica membranes. The desalting of the membrane is done in an additional filtration step with a wash buffer. After the DNase treatment of the samples, which is done directly on the membrane during 15min at room temperature, the silica membranes are washed three times with three different wash buffers. Once the binding plate is dry, the very pure total RNA is eluted with 50-130µl RNase-free water. As an option, a clog check for the filter plates after every vacuum step can be selected.

Validation

The MICROLAB® STAR is validated for the automation of the MACHEREY-NAGEL NucleoSpin® 96 RNA kit. The validated system includes the instrument, the labware carriers and the software.

Results

Samples of 10mg guinea pig liver are manually homogenized in 130µl lysis buffer and prefiltered in a centrifuge. The lysates are then placed on the deck of the MICROLAB® STAR in Eppendorf tubes for the isolation of RNA following the method described above.

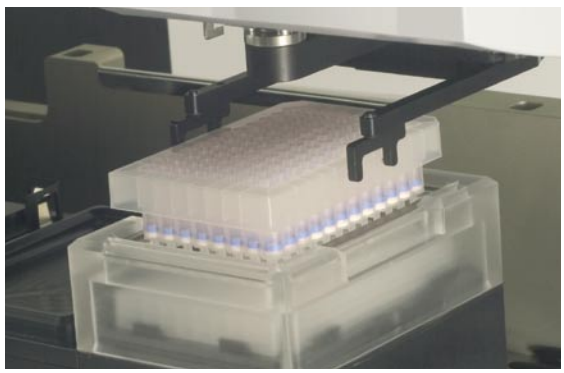

Figure 1: *Assembling and disassembling of the BVS vacuum system is performed by the iSWAP robotic plate-handler.*

HAMILTON

For the isolation of RNA out of HT-29 colonic epithelial cells, $5x10^5$ cells are lysed in 400 µl lysis buffer RA1 and then placed on the deck in Eppendorf tubes for the RNA isolation. Alternatively, homogenization and lysis of tissues as well as lysis of cells can be performed in suitable 96-well plates.

After the isolation of RNA was finished from the liver tissue and from cells, the yields were measured with a fluorescence assay (Ribogreen) on a SpectraMax Reader from Molecular Devices. The quality of the total RNA was analyzed using an Agilent Bioanalyzer 2100.

Homogenous RNA quality and yields

Isolated total RNA were analyzed for quality using the Agilent Bioanalyzer 2100. Homogenous results were obtained with RNA isolated from HT-29 colonic epithelial cells and guinea pig liver. (Figure 2). The ratio between 28S rRNA and 18S rRNA of the isolated total RNA was always within the optimal range of 1.6 and 2.0.

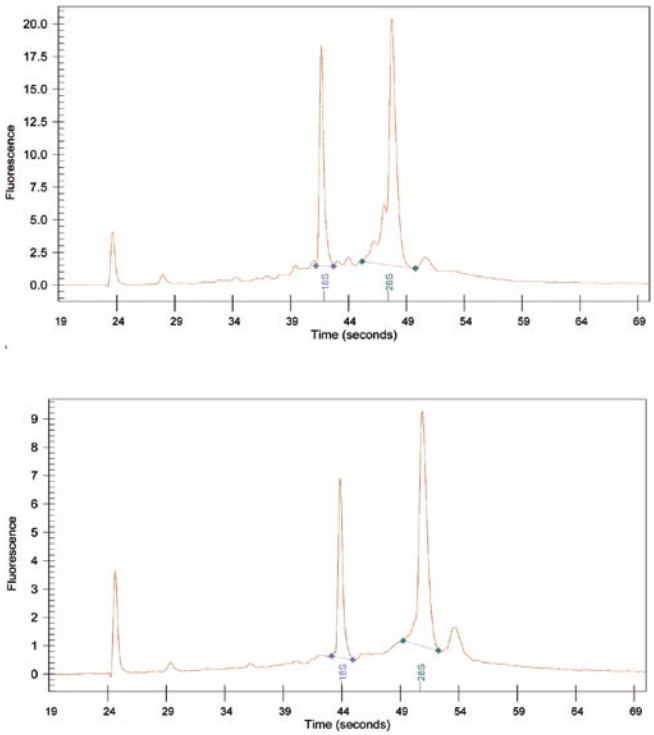

Figure 2: *Quality control with Agilent Bioanalyzer 2100 of isolated total RNA from a) 10 mg liver tissue b) 5x10⁵ HT-29 colonic epithelial cells with NucleoSpin® 96 RNA Kit on MICROLAB® STAR. The ratio between 28S rRNA and 18S rRNA is within the optimal range of 1.6 and 2.0.*

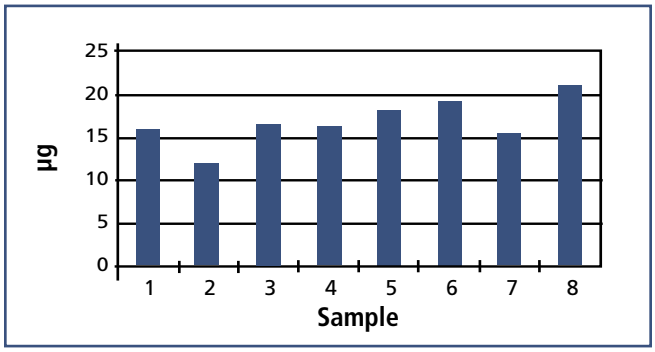

Figure 3: *Quantification of total RNA with Ribogreen assays. RNA was isolated from 10mg liver tissue from guinea pigs. The isolated amounts of RNA range from 12µg to 21µg.*

The yields were defined with a Ribogreen assay. Starting with 8 different samples of 10mg guinea pig liver, the isolated amounts of total RNA were between 12µg and 21µg (Figure 3).

Throughput and Capacity

The isolation of 96 RNA samples with the NucleoSpin® Robot-96 RNA kit was completed in 60 minutes. This system can process up to four 96-well plates in 4 hours without any user intervention. Deck capacity – and therefore walk-away time – may be increased by integrating additional plate stackers.

Discussion

HAMILTON and MACHEREY-NAGEL have developed a validated method for fully automated total RNA isolation out of cells or tissue with maximum reliability, yield and quality. The highly flexible system provided by HAMILTON can be adapted to other MACHEREY-NAGEL kit types. Further options such as RNA normalization, cDNA synthesis and RT-PCR are also available.

Features and Benefits

- Fully automated hands-free processing with built-in robotic plate-handler (iSWAP)
- Usage of disposable tips with filters guarantees contamination-free processing
- Optional clog check for monitoring of the vacuum steps
- Same method for RNA isolation out of cells and tissue
- Automation of additional applications like RNA normalization, cDNA synthesis and RT-PCR

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