MACHEREY-NAGEL

NucleoSpin® 8 RNA

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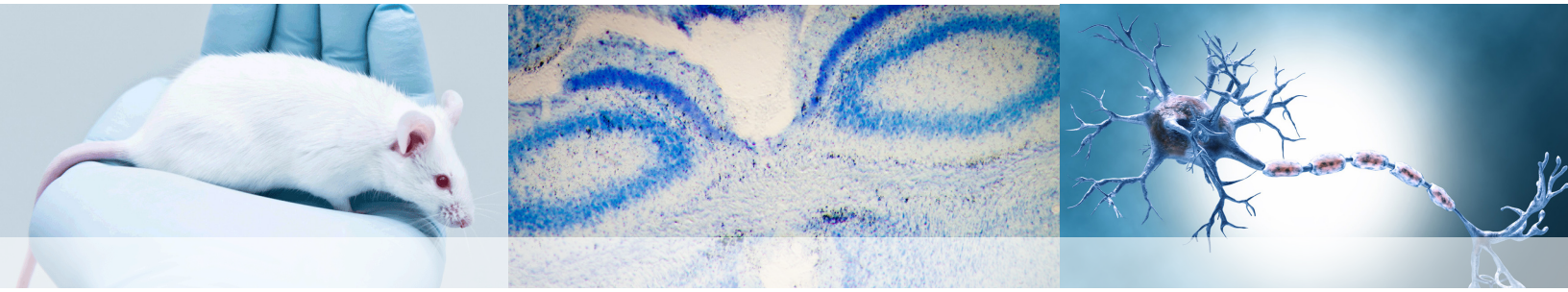
Automated RNA purification from mouse brain tissue using the MACHEREY-NAGEL NucleoSpin® 8 RNA kit on the Eppendorf ep*Motion*® 5075 TMX



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Abstract

Multiple sclerosis is a chronical inflammatoric disease of the central nervous system in which the body's immune response attacks the nerve cells, leading to demyelination. In a mouse model system demyelination can be triggered by cuprizone. In the present application note the influence of cuprizone on the demyelination and the invasion of astrocytes and microglia cells in the affected brain areas is investigated. Total RNA from different brain tissue samples was isolated manually with the MACHEREY-NAGEL NucleoSpin® RNA/Protein kit, or with the NucleoSpin® 8 RNA kit automated on an ep*Motion*® 5075 TMX liquid handler. Results about the yield and quality of extracted RNA are presented as well as use of the RNA in down stream RT-PCR.

Introduction

Multiple sclerosis (MS), also known as encephalomyelitis disseminata (ED) is a chronical inflammatoric disease of the central nervous system in which the body's immune response attacks the nerve cells, leading to demyelination. As the demyelination can be triggered in mice, this serves as a model system for the investigation or regulatory processes in the onset of MS. By feeding the copper chelator cuprizone a significant demyelination can be induced. The readjustment to cuprizone-free nutrition leads to a complete remyelination. In the present study the influence of cuprizone on the demyelination and the invasion of astrocytes and microglia cells in the affected brain areas is investigated. Histological changes are correlated with gene expression data. For a reliable gene expression study it is mandatory to isolate RNA of high purity and quality. The RNA isolation from lipid rich brain tissue is still challenging. Furthermore, high throughput sampling is a time consuming procedure. In this study total RNA from different brain tissue samples was isolated manually with the MACHEREY-NAGEL NucleoSpin® RNA/Protein kit, or with the NucleoSpin® 8 RNA kit automated on an ep*Motion*® 5075 TMX liquid handler.

Material and methods

- Eppendorf ep*Motion*® 5075 TMX
- Vac Frame 2
- Vac Holder
- Reservoir 400 mL
- Collection Plate Adapter for MN Tube Strips
- Channeling Plate
- Reservoir Rack with Reagent Reservoirs
- NucleoSpin® 8 RNA kit
- Mouse brain tissue samples

Demyelination was induced in C57BL/6 mice by feeding a diet with 0.2–0.4 % cuprizone. After 1–8 weeks the brains were dissected and sectioned with 5 µm thickness for histological/immunohistochemical analysis. For gene expression analysis, mice were killed and the investigated brain parts of the basal ganglia system were dissected on the level of commissural anterior. Tissues were immediately snap frozen in liquid nitrogen and kept at -80 °C until further use. For RNA isolation the tissue samples were homogenized with a Precellys 24 homogenizer. For manual RNA isolation total RNA was isolated with the NucleoSpin® RNA/Protein Kit. For automated processing the NucleoSpin® 8 RNA kit was used on an ep*Motion*® 5075 TMX liquid handler. Automated processing was done according to the procedure described in the NucleoSpin® 8/96 RNA kit user manual with the ep*Motion*® Plug-*N*-Prep method for NucleoSpin® 8 RNA kit. RNA quality was determined by analysis on the Agilent Bioanalyzer 2100 and RNA Nano assays. Isolated RNA was quantified by A_{260} measurement, for the evaluation of RNA purity the ratios A_{260}/A_{280} were determined. Subsequent gene expression analysis was done after reverse transcription using the Invitrogen M-MLV RT kit. Relative quantification of the astrocyte specific GFAP gene (glial fibrillary acidic protein) was performed using the DCt method which results in ratios between target genes and a housekeeping reference gene (HPRT, hypoxanthin guanin phophoribosyl transferase).

Table 1: ep*Motion*® 5075 TMX worktable details for NucleoSpin® 8 RNA protocol

Position	Labware	Comment
A2	MN Tube Strips	Elution tubes
A3	epT.I.P.S Motion 1000 µL	
A4		TMX shaker
B1	epT.I.P.S Motion 1000 µL	
B2	epT.I.P.S Motion 300 µL	
B3	Reagent Reservoirs: Position 1: Buffer RA1 Position 2: Buffer RA4 Position 3: Buffer RA3 Position 4: empty Position 5: Buffer RA2 Position 6: water Position 7: DNase solution	Reservoir size: 100 mL 100 mL 100 mL – 100 mL 30 mL 30 mL
Vacuum	NucleoSpin® RNA Binding Strips Vacuum Frame 2 Reservoir 400 mL with channeling plate	RNA binding strips inserted into Column Holder A (top) collar for vacuum manifold collects waste
C3	2.1 mL deep-well plate	Samples
C4	Vacuum Frame Holder	Height adapter for vacuum Frame 2
T0	Gripper	
T1	TM 1000-8	1000 µL 8-channel pipetting tool

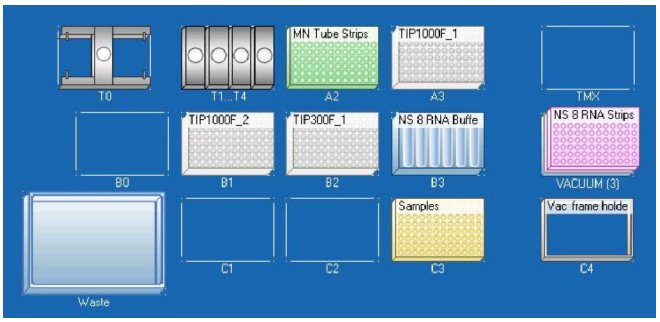

Figure 1: ep*Motion*® Editor

Screenshots from the ep*Motion*® Editor showing the setup of the ep*Motion*® 5075 TMX worktable for use with the NucleoSpin® 8 RNA kit.

Results

The administration of cuprizone induced a highly reproducible demyelination of distinct basal ganglia system subregions (Fig. 1). Fig. 2A shows a hematoxylin and eosin-stained forebrain on the level of the basal ganglia system (CNH = head of caudate nucleus, CNV = ventral part of caudate nucleus, CP = caudate-putamen, GP = globus pallidus.

The line shows the visual separation of the basal ganglia system into a lateral and medial part.

Intact myelin in LFB-stained (luxol fast blue) sections appears as blue fiber tracts (Fig. 2B-G). Cuprizone exposure resulted in a massive demyelination as detected by a loss of LFB-stained fibers in different white matter tracts compared to controls.

It is well known from other brain regions that astrocytosis and microglia cell invasion and/or proliferation occurs during cuprizone induced acute demyelination. As can be seen in Fig. 3 a significant invasion of astrocytes and microglia cells is observed after cuprizone administration. While under control conditions the astrocytes revealed thin cells only with a few short processes (Fig. 2 a-b), after cuprizone treatment the astrocytes became hypertrophic and multipolar (Fig. 2 c-d).

Quantification of astrocyte numbers also correlated with the previous cuprizone treatment: in the medial brain part cell numbers were 6 +/-2 and 2 +/- 0.5 cells/mm^2 for control and cuprizone-treated animals,

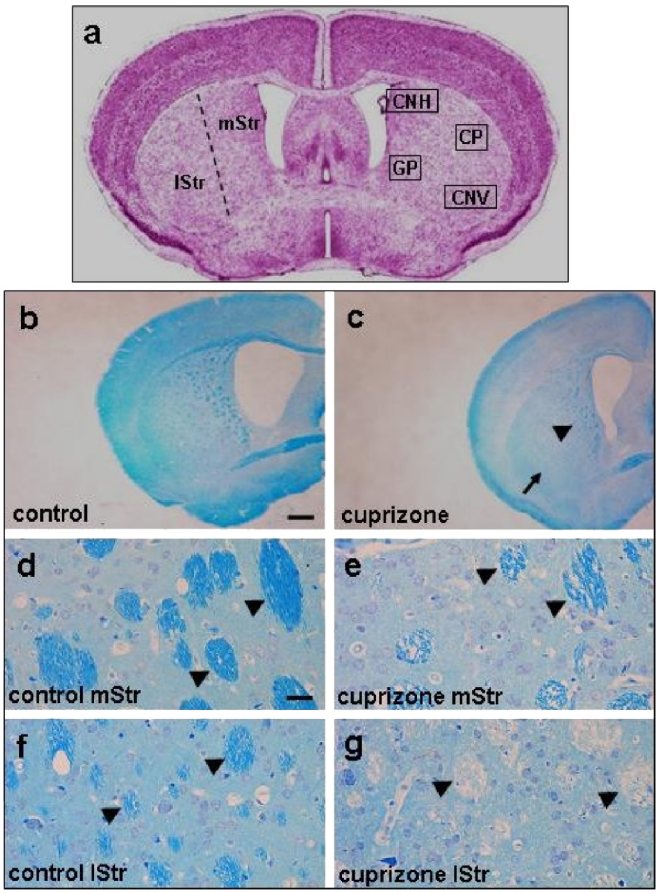

Figure 2: Stained brain tissue.

a) Hematoxylin and eosin-stained forebrain on the level of the basal ganglia system (CNH = head of caudate nucleus, CNV = ventral part of caudate nucleus, CP = caudate-putamen, GP = globus pallidus. b)-g): LFB-stained (luxol fast blue) brain sections. For further explanation please refer to the text.

in the lateral areas the cell numbers were 90 +/- 6, and 70 +/- 14 cells/mm^2, respectively (Fig. 3 g).

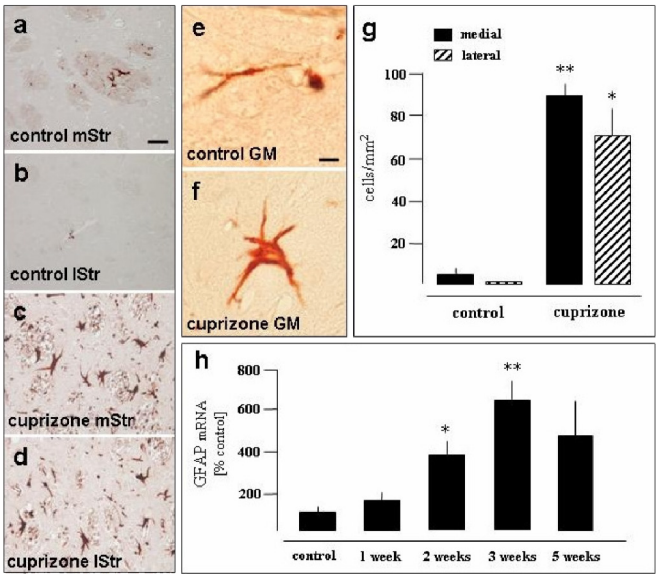

Figure 3: a)-d): Invasion of astrocytes and microglia cells after cuprizone administration, control a)-b), after cuprizone treatment c)-d). g): number of astrocytes after cuprizone treatment. e)-f): morphology of astrocytes with/without cuprizone treatment. h) expression of GFAP depending on the time after cuprizone treatment.

Parallel to the histological investigations total RNA was isolated from the investigated brain areas and subjected to further analysis. The mean ratio A_{260}/A_{280} was 1.92 for manual processing with the NucleoSpin RNA/Protein kit and 1.96 for the automated procedure on the ep*Motion*®. Ratios A_{260}/A_{228} were > 1.96 in all cases, indicating RNA of excellent purity. On the Bioanalyzer the total RNA appears as two distinct peaks for the 18S and 28S ribosomal RNA (Fig. 4).

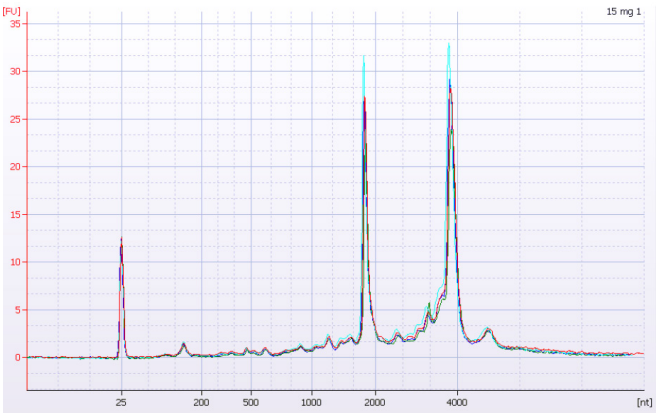

Figure 4: Electropherogramm of isolated RNA.
Agilent Bioanalyzer 2100 with RNA Nano 6000 Assay. RNA was isolated from 15 mg of brain tissue.

RIN values between 8 and 9 were obtained for different samples and different sample amounts. A summary of RIN values is given in Table 2. Obviously, RNA of highest quality and integrity was isolated from the difficult brain tissue, meeting the requirements of even the most demanding downstream applications such as RT-PCR or Affymetrix analysis.

Table 2: High RIN values.
Summary of RIN values and RNA concentration as determined from several samples with the Agilent Bioanalyzer 2100 and RNA Nano 6000 assays. Mean values of 8 replicates each.

Sample	5 mg		10 mg		15 mg	
	ng/µL	RIN	ng/µL	RIN	ng/µL	RIN
	30	8.0	70	8.6	82	8.8
	32	8.8	68	8.6	78	8.9
	35	8.8	63	8.3	93	8.6
	33	8.7	75	8.6	89	8.7
mean	32.5	8.6	69	8.5	85.5	8.8

Total RNA yield corresponded well with the tissue amount used for extraction. From 5, 10 and 15 mg of brain tissue, 3.5, 7.4 and 9.4 µg RNA, respectively, were obtained (photometric quantification).

Subsequently, isolated RNA was subjected to gene expression analysis. In Fig. 3 h the expression of GFAP depending on the time after cuprizone treatment is shown. As can be seen the maximum GFAP expression was observed at 3 weeks after cuprizone treatment, which correlates well with the histological data from the astrocyte cell numbers.

Summary

In summary, total RNA in high quality and excellent integrity could be isolated from several brain tissue samples either manually or automated on the ep*Motion*® 5075 TMX liquid handler. Gene expression analysis data after quantitative RT-PCR correlated well with histological findings. Although very demanding lipid rich brain tissue was used as sample material the RNA isolation procedure was robust and reproducible allowing for very consistent quantification of gene expression levels.

References

[1] Eppendorf Application Note 186: Automated RNA purification in 96-well plate and 8-well strip format from human cells or animal tissue using the MACHEREY-NAGEL NucleoSpin®8/96 RNA kits on the ep*Motion*® 5075 from Eppendorf

Plug-n-Prep Method file available at www.epmotion.com/pnp

Ordering Information

Product	specifications	Preps	REF
NucleoSpin® 8	Kit based on silica membrane technology for the isolation of RNA from tissue samples.	12 x 8 / 60 x 8	740698 / .5
NucleoSpin® 96	Kit based on silica membrane technology for the isolation of RNA from tissue samples.	2 x 96 / 4 x 96 / 24 x 96	740709.2 / .4 / .24
Starter Set A	For use of NucleoSpin® 8-well strips on the NucleoVac 96 Vacuum Manifold		740682