Purification kits, RNA, NucleoSpin® RNA II

LE

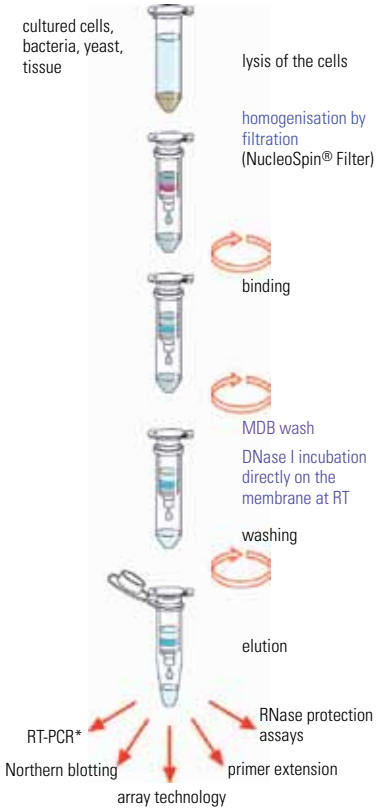
MACHEREY-NAGEL

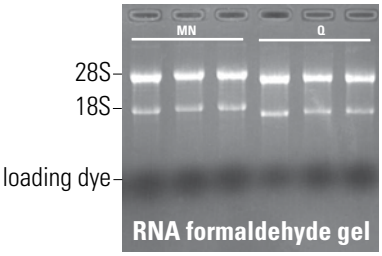
For total RNA isolation from cultured cells, tissue, bacteria, yeast, biological fluids, samples stored in RNAlater, saliva samples and RNA clean up from reaction mixtures.

NucleoSpin® RNA II is recommended for isolation of total RNA from cultured cells, tissue, bacteria, yeast, cell-free biological fluids and reaction mixtures. This kit allows purification of up to 70µg of highly pure, essentially DNA-free total RNA.
As reference RNA from 10^6 cultured HeLa cells is prepared following standard protocol resulting in 15µg to 20µg, but at least 10µg of total RNA. It is possible to detect transcripts from very low amounts of cells. Total RNA prepared with NucleoSpin® RNA II is suitable for applications like reverse transcriptase-PCR* (RT-PCR*), Northern blotting, primer extension, array technology or RNase protection assays.
For isolation of high quality RNA it is important to prevent degradation of the RNA and to eliminate genomic DNA. With the NucleoSpin® RNA II method, cells are lysed by incubation in a solution containing large amounts of chaotropic salts. This lysis buffer immediately inactivates RNases – which are present in virtually all biological materials – and creates appropriate binding conditions which favour adsorption of RNA to the silica membrane. After lysis, homogenisation and reduction of viscosity are achieved by filtration with NucleoSpin® filter units.
Contaminating DNA bound to the silica membrane is removed by DNase I solution which is directly applied onto the silica membrane during the preparation. Optimal conditions for the DNase I are achieved by washing the silica membrane with a specific salt buffer before treatment. Salts, metabolites and macromolecular cellular components are removed by simple washing steps with two different buffers. Total RNA is finally eluted with RNase-free water. Sharp rRNA bands show high structural integrity.
Treatment of RNA eluates with RNase A shows efficient removal of genomic DNA in NucleoSpin® RNA II preparations. Truly quantitative removal of genomic DNA is shown by PCR* control experiments using different sensitive gene-specific primers. Hands-on time for total RNA preparation from cultured cells or tissue with NucleoSpin® RNA II is less than 30 minutes.

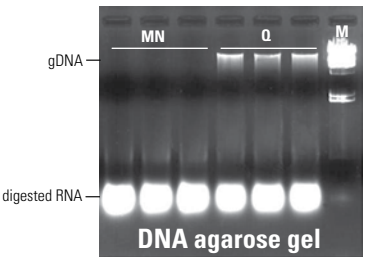
Kit components: NucleoSpin® RNA columns with collecting tubes, collecting tubes 2mL, microcentrifuge tubes 1.5mL, NucleoSpin® filters, buffers, RNase-free DNase I, DNase I reaction buffer, RNase-free water.

NucleoSpin® RNA II procedure

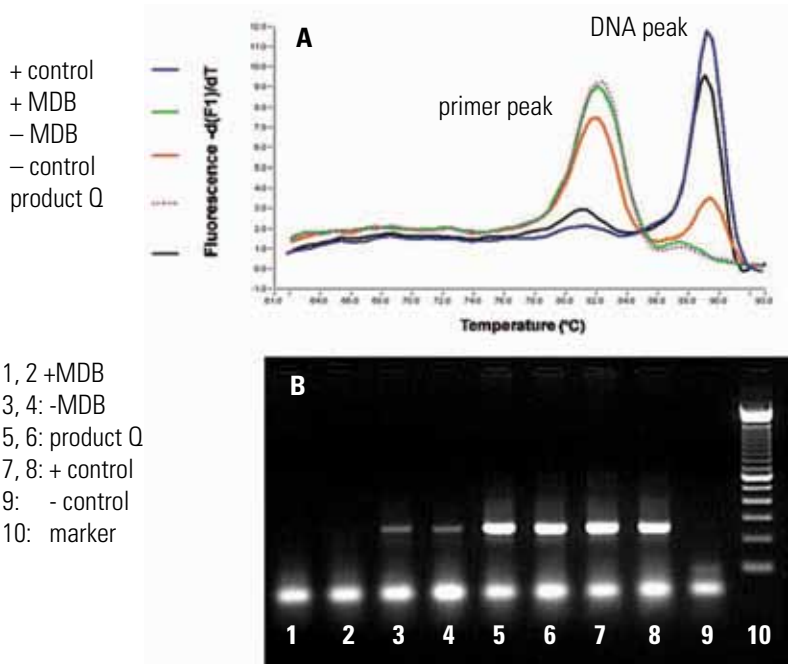




A: Total RNA was purified from 10^6 HeLa cells using the NucleoSpin® RNA II kit in comparison to competitor Q. 10µL of each eluate (elution volume 50µL) were analysed on a 1.2% formaldehyde gel. The $A_{260/280}$ was on average 2.1. Due to lower detection sensitivity of genomic DNA in a denaturing formaldehyde gel contaminating DNA is often not visible.



B: For visualisation of residual genomic DNA 10µL of each eluate were treated with RNase A and subsequently loaded onto a 1% TAE agarose gel. The NucleoSpin® RNA II kit contains DNase I for on-column DNA digestion.
M: λ*Hin*dIII, MBI-Fermentas
Optimised DNase I treatment eliminates genomic DNA in NucleoSpin® RNA II preparations, whereas a clear distinct band of contaminating genomic DNA is visible in RNA samples prepared with a kit of competitor Q.



Optimised DNase I digest by use of MDB (membrane desalting buffer)
Total RNA was purified using NucleoSpin® RNA II with (+MDB) or without (-MDB) use of membrane desalting buffer prior to DNase I digest. DNA contamination of purified RNA samples was determined by LightCycler™ analysis (A). Corresponding PCR* products were separated by agarose gel electrophoresis (B).
No genomic DNA is detected if MDB is applied.