NucleoSpin® RNA – isolation of RNA from difficult-to-lyse tissue (Rev. 01)

This protocol is only a supplement to the kit's general user manual. Please refer to the kit manual for more detailed information regarding safety instructions, product-specific disclaimers, and especially preparations needed before starting the procedure. The latest version of the user manual is available at ***www.mn-net.com/usermanuals*** *or can be requested from our technical service (tech-bio@mn-net.com). Material safety data sheets (MSDS) can be downloaded from* ***www.mn-net.com/MSDS****.*

This support protocol has been developed for RNA isolation from 'difficult' tissues using the NucleoSpin® RNA kit to improve RNA yield and quality when isolating RNA from difficult-to-lyse tissues (e.g., cerebellum, heart, or skeletal muscle).

1	Add **400 µL Buffer RA1** to the sample. Homogenize with NucleoSpin® Filter, homogenizer, or syringe / needle method.
2	Centrifuge at **14,000 x *g*** for **5 min** to remove debris.
3	Carefully transfer supernatant to a new 1.5 mL tube. Avoid pipetting pelleted material.
4	Add **300 µL ethanol (96–100 %)** to the sample, mix very well by vortexing.
5	Centrifuge at **14,000 x g** for **10 min**. Discard as much supernatant as possible.
6	Air-dry **5 min.**
7	Add **25 µL RNase-free water** to the pellet and resuspend completely.
8	Add **375 µL Buffer RA1**, mix by pipetting and light vortexing.

Continue with step 4 of the standard protocol.