TROUBLESHOOTING

Problem	Possible cause	Solution
RNA Purification Column becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Tissue and cell debris were transferred into the column.	Pipette the supernatant carefully, without disturbing the tissue or cell pellet.
	The purification column is overloaded.	Do not exceed 30 mg of tissue and 10^7 cells during purification.
Low RNA yield.	Tissue was incorrectly stored or preserved: RNA degradation.	Store tissue at -80°C no longer than a year. If tissue storage buffer was used, ensure if it was of a good quality and that the storage conditions were adequate.
	Too little sample material was used.	Take more sample material. A proper amount of the material is dependent on the kind of a cell line/tissue examined and needs to be optimized individually.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in RLys Buffer. A tissue must be first fragmented into smallest possible pieces and homogenized by an appropriate method.
	Inefficient homogenisation due to an excessive foaming.	Add Antifoam Reagent to RLys Buffer. Mix AF Reagent before use and pipette it carefully due to a high viscosity.
	The purification column has become clogged.	See "RNA Purification Column becomes clogged during purification".
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA is still bound to the column membrane.	Repeat the RNA elution.
Low purified RNA concentration.	Too much of elution buffer was used.	Decrease the REB volume to 30–50 µl. For a sample concentration it is possible to reload the eluate onto the column and centrifuge again.
Too low A_{260}/A_{230} ratio of purified RNA.	Remainings of buffers present in the eluate.	Ensure that the purification column had been properly dried before elution and no droplets remained on the ring. If necessary, increase centrifugation speed at step 8 of Isolation Protocol (section XI) to 18 000 x g. Carefully remove the column from a collection tube.
	Incomplete sample loading.	Make sure that lysate has passed completely through the RNA Purification Column before proceeding through washing steps. If necessary, increase centrifugation speed at step 4 of Isolation Protocol (section XI).

Purified RNA is degraded.	Old material was used.	Performing an isolation from fresh tissues is recommended.
	Material was repeatedly frozen/thawed.	Avoid subjecting the sample material to repeated freeze/thaw cycles.
	RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
	RNA degraded as a result of over-intensive homogenization.	The recommended homogenization conditions should be applied (see section IXA).
DNA contamination present.	Too much sample material was used.	Decrease the amount of a sample material. Optionally, the purified RNA sample may be treated with DNase.
	High amount of DNA present in a material.	The use of on-column DNA digestion is recommended during the RNA purification procedure. While isolating from high amount of material or particular sample type (e. g. brain), incubation with DNase can be prolonged to 15 minutes.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied (see section IXA).
	DNase is inactive.	Prepare fresh DNase solution. Ensure that DNase solution is stored as recommended.