XII. TROUBLESHOOTING

Problem	Possible cause	Solution
Column H becomes clogged during purification.	Inappropriate tissue homogenization.	Select the appropriate homogenization conditions (see section IXA).
	Tissue and cell remains were transferred into the column H.	Pipette the supernatant carefully, without disturbing the tissue or cell pellet.
	The purification column is overloaded.	See "Column H becomes clogged during purification".
Low RNA isolation efficiency.	Tissue was incorrectly stored or preserved: RNA degradation.	Store tissue at -80°C no longer than a year. If a tissue storage buffer was used, ensure its good quality and that the storage conditions are adequate.
	Too little sample material was used.	Take more sample material.
	Insufficient fragmentation of the sample material.	Ensure proper tissue homogenization in the RLys Buffer. The tissue must be first fragmented into as the smallest possible pieces and homogenized by an appropriate method.
	The purification column has become clogged.	See "Column H becomes clogged during purification".
	The RNases are present.	See "RNase elimination" in section VIII. Recommendations and Important Notes.
Low purified RNA concentration.	Too much of the elution buffer was used.	Decrease the REB volume to ≥5 µl.
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.
	The 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 IX).
Low purified RNA concentration.	Too much of the elution buffer was used.	Decrease the REB volume to ≥5 µl.
DNA contamination present.	Too much sample material was used.	Decrease the amount of sample material. Optionally, the purified RNA sample can be treated with a DNase.
	Inappropriate homogenization.	The recommended homogenization conditions should be applied.
	DNase is inactive.	Prepare a fresh DNase solution. Ensure that the DNase solution is stored as recommended.