

XIII. TROUBLESHOOTING

Problem	Possible cause	Solution
Incomplete cell wall lysis.	Too many cells were taken for RNA isolation.	Reduce the amount of starting material.
	No reducing agent added.	Repeat isolation, ensuring that the appropriate quantity of β -mercaptoethanol has been added.
	BACTERIA: Gram-positive strain was taken for DNA isolation.	Follow the instruction regarding the RNA isolation from Gram-positive bacteria (section IX. Sample preparation).
	YEAST: Reduced efficiency of RNA Yeast Lysis Mix.	Use fresh RYLM Buffer. Ensure that RYLM Buffer is stored at -20°C .
Column H or B becomes clogged during purification.	Incomplete cell lysis.	See "Incomplete cell wall lysis".
	The purification column is overloaded.	Repeat the centrifugation step for 60 s at $21\,000 \times g$ or at the maximum speed.
Low RNA isolation efficiency.	Starting material contained few bacterial or yeast cells.	Increase the amount of starting material or decrease the volume of RNA Elution Buffer.
	Incomplete cell lysis.	See "Incomplete cell wall lysis".
	The purification column has become clogged.	See "Column H or B 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 Elution Buffer was used.	Decrease the volume of RNA Elution Buffer to 30-50 μL .

Purified RNA is degraded.	Old material was used.	It is recommended to perform an isolation from fresh overnight bacterial or yeast broth culture.
	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.
Isolated RNA is of poor purity.	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
DNA contamination present.	Too much starting material.	Decrease the amount of sample material.
	DNase is inactive.	Prepare a fresh DNase solution. Ensure that the DNase solution is stored as recommended.