



NanoCinna

Pharmacogenomics Center

DNA/RNA Extraction

Troubleshooting guide

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Troubleshooting

If any problems with the preparation arise proceed as follows: In order to get an idea of what has been the problem, please collect the flow-through, the wash and the eluate fraction. Precipitate the fractions and load them on an agarose gel. In combination with this troubleshooting guide this will help to solve your problem.

Problem	Possible cause	solution
Inefficient disruption and/or homogenization		Increase <i>g</i> -force and centrifugation time if necessary. In subsequent preparations, reduce the amount of starting material and/or increase the homogenization time.
Incomplete removal of cell-culture medium		When processing cultured cells, ensure complete removal of cell-culture medium after harvesting cells.
Too much starting material		Reduce the amount of starting material. It is essential to use the correct amount of starting material.
Low yield of DNA	<p>Sample was frozen and thawed several times. Sample was stored at 2-4°C longer than 2 months. Blood clots were present in the sample.</p> <p>Too much cells were used for purification, and DNA pellet turned insoluble.</p> <p>DNA pellet was not completely dissolved. Large DNA molecules will not be quantitatively recovered if the DNA pellet is not completely dissolved.</p>	<p>Take a new sample.</p> <p>Reduce cell quantity by a factor of two or more. Take a new sample.</p> <p>Remove supernatant completely (do not dry) and dissolve DNA pellet completely in appropriate solution by gentle vortexing. Make sure that the pellet is completely dissolved.</p>
Degraded DNA	Inappropriate storage conditions of the sample.	<p>Ensure that tissue samples are properly stabilized and stored in RNA Stabilization Reagent.</p> <p>For frozen cell pellets or frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the DNA/RNA procedure quickly, especially the first few steps.</p>

DNA fragmented	Homogenization too vigorous.	The length of the purified DNA (usually 15–30 kb) depends strongly on the homogenization conditions. If longer DNA fragments are required, keep the homogenization time to a minimum or use a gentler homogenization method if possible.
DNA contaminated with RNA	Lysate applied to the DNA spin column contains ethanol. Sample is affecting pH of homogenate.	Add ethanol to the lysate after passing the lysate through the DNA spin column. The final homogenate should have a pH of 7. Make sure that the sample is not highly acidic or basic.
Column blocked	Viscosity of the sample is too high.	Use larger buffer volumes for sample preparation. Use a prolonged centrifugation step to get a clear supernatant.
Low RNA yield	Too much starting material was used for lysate preparation. Incomplete disruption and homogenization of sample. Ethanol was not added to the lysate. Over dried RNA pellet. Final RNA pellet incompletely redissolved. Large RNA molecules will not be quantitatively recovered if the RNA pellet is not completely dissolved.	Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocol. Reduce the amount of starting material. Increase the duration and intensity of sample disruption. Make sure that ethanol was added to the lysate before applying the sample to the purification column. Do not use a Speed Vacuum to dry the pellet. Dry pellet briefly at room temperature for 5 minutes. Add DEPC treated water to the tube and pipet up and down several times to resuspend Remove supernatant completely (do not dry) and dissolve RNA pellet completely in appropriate solution by gentle vortexing. Make sure that the pellet is completely dissolved.
RNA degraded	Inappropriate handling of starting material.	Ensure that tissue samples are properly stabilized and stored in RNA Stabilization Reagent. For frozen cell pellets or frozen

<p>RNA degraded</p>	<p>RNase contamination of solutions supplied by the user.</p> <p>RNase contamination of plasticware and work station.</p> <p>Cells were dispersed by trypsin digestion.</p>	<p>tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at – 70°C. Perform the DNA/RNA procedure quickly, especially the first few steps.</p> <p>Although all buffers have been tested and are guaranteed RNase-free, RNases can be introduced during use. Be certain not to introduce any RNases during the DNA/RNA procedure or later handling.</p> <p>Maintaining an RNase-Free Environment.</p> <p>If trypsin is used for detach the cells, inactivate it before proceeding (typically this is done by rinsing in 1X PBS and resuspending in culture medium).</p>
<p>Contamination of RNA with DNA</p>	<p>Cell number too high.</p> <p>Incomplete removal of cell-culture medium or stabilization reagent.</p> <p>Tissue has high DNA content.</p> <p>Samples used for the isolation contained organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline solution.</p>	<p>For some cell types, the efficiency of DNA binding to the AllPrep DNA spin column may be reduced when processing very high cell numbers. If the eluted RNA contains substantial DNA contamination, try processing smaller cell numbers.</p> <p>Be sure to remove any excess cell-culture medium or stabilization reagent to prevent significant dilution of the lysis buffer.</p> <p>For certain tissues with extremely high DNA content (e.g., thymus), some DNA will pass through the DNA spin column. Try using smaller samples.</p>

Insufficient resuspension of purified nucleic acid	Nucleic acid was over dried. Residual salt or organic solvent in the pellet.	Dissolve for a longer time at somewhat higher temperature. Wash the pellet with an organic solvent of low viscosity. Increase buffer volume.
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1. www.fermentas.com
2. <https://commerce.invitrogen.com/index.cfm?fuseaction=iProtocol.unitSectionTree&objectId>
3. http://www.ambion.com/techlib/prot/fm_1722.pdf

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