

JETQUICK Tissue DNA Kit

Extended Troubleshooting Guide

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Problem: Low DNA yield	
<u>Problem</u>	<u>Help and comments</u>
DNA yield from tissue sample is lower than expected (general notes)	<p>The DNA content of different tissue materials can be very different, e.g. muscle tissue contains less DNA than spleen tissue.</p> <p>If more DNA is required from a tissue that doesn't contain much DNA, increase the amount of starting material. In such a case the volumes of all components used to solubilize the tissue (buffer T1, proteinase K and optionally RNase A) must be scaled up proportionally. In such a case, multiple rounds of spin-column loading are required.</p>
Tissue lysis (general notes)	<p>Make sure, that the tissue sample is lysed efficiently by treatment with proteinase K and buffer T1.</p> <p>If possible, cut down the tissue sample to small pieces or grind the sample under liquid nitrogen with a pestle in a mortar. Incubate at 56 °C. It will take some time to solubilize the tissue sample completely. Vortex-mix the sample in buffer T1 a couple of times during incubation. Repeated vortexing will facilitate solubilization of the tissue sample.</p> <p>If mixing with buffers T1 and later T2 was insufficient, remaining cellular matter may clog the spin column and subsequent DNA yield will be low. In such a case repeat the DNA purification with new samples.</p>
Storage of tissue samples	For long-term storage tissue samples are optimally kept at -70 °C to -80 °C. Lower yields will be obtained from material that has been inappropriately stored.

Incubation with buffer T1 and proteinase K	<p>Too short incubation times with buffer T1/proteinase K may lead to insufficient lysis, causing clogging of the spin column and subsequently low DNA yield.</p> <p>Time to reach complete solubilization of the tissue sample varies significantly between different tissue types. If necessary, either extend incubation time or increase the amount of proteinase K two-fold to obtain complete lysis.</p> <p>Some parts (e.g. hair and bones from mouse tails) will not dissolve readily upon treatment with buffer T1/proteinase K. If there is still undissolved matter present after an overnight incubation at 56 °C, spin down solid particles for 5 min. at max speed and process only the clear supernatant in the next step.</p>
	<p>Make sure, that no white precipitate has appeared in buffer T1 before addition of the buffer to the sample.</p> <p>If a precipitate is present in buffer T1, re-dissolve it by incubating the buffer bottle for a few minutes in a water bath at 50 °C with swirling the bottle from time to time.</p>
Check proteinase K activity	<p>Proteinase K activity may decrease with time and in particular after repeated freeze/thaw cycles. If proteinase K doesn't work any longer as expected, prepare new enzyme solution from fresh stock.</p> <p>A decreased enzyme activity may lead to insufficient tissue lysis, thus causing low DNA yields.</p>
Mixing with buffer T2	<p>Tissue lysate must be mixed to homogeneity with buffer T2. As buffer T2 is quite viscous, mixing should be carried out by pulse-vortexing using multiple pulses.</p> <p>Simply mixing by inverting may not be sufficient to obtain the homogeneity, that is necessary for optimal DNA yield and purity.</p>
Mixing with absolute ethanol	<p>Addition of absolute ethanol is mandatory to decrease foam and achieve optimal DNA yield and purity.</p> <p>Mix the sample immediately and very thoroughly by pulse-vortexing after adding the ethanol to avoid too high local alcohol concentrations.</p>
Washes	<p>Make sure, that wash buffers TX and T3 are reconstituted properly with <u>absolute ethanol</u> as outlined on the respective bottle's label.</p> <p>Use wash buffers TX and T3 in the correct order as stated in the protocol (TX before T3).</p>

Elution	<p>Using pre-warmed (65-70°C) elution buffer is highly recommended. Elution buffer at room temperature may also lead to satisfactory results, but elution efficiency is significantly higher with warm elution buffer.</p> <p>After having pipetted elution buffer onto the silica membrane, incubate for at least 1 min (better 5 min) at room temperature. Shorter incubation times may lead to a reduced DNA recovery.</p>
	<p>Elution buffer should have a slightly basic pH (8.0 – 9.0) for optimal elution efficiency. Using plain water with a pH at <7.0 may lead to a decreased DNA yield.</p>
Viscosity of the lysate is high	<p>If the tissue sample contains much DNA, the lysate may become very viscous due to the release of quite high amounts of high-molecular weight DNA.</p> <p>To reduce viscosity, DOUBLE amounts of buffer T1, T2 and absolute ethanol. Mix immediately and thoroughly.</p> <p>This may require multiple rounds of loading the respective spin column.</p>

Problem: Low DNA concentration in the eluate	
<u>Question / Problem</u>	<u>Help and comments</u>
DNA concentration in the eluate is too low.	<p>DNA yield depends on the amount of DNA in the tissue sample. If DNA content of the tissue sample is low, DNA yield and concentration in the eluate will be low, too.</p> <p>If more DNA is required from a tissue that doesn't contain much DNA, increase the amount of starting material. In such a case the volumes of all components used to solubilize the tissue (buffer T1, proteinase K and optionally RNase A) must be scaled up proportionally. In such a case, multiple rounds of spin-column loading are required.</p>
	<p>For samples where a low DNA yield is to be expected, elution volume can be reduced by 50 – 75%, e.g. DNA from a tissue miniprep can be eluted with 50 – 100 µl of elution buffer instead of 200 µl as outlined in the standard protocol.</p>
	<p>Precipitate the DNA from the eluate by adding 1/10 vol. 3 M sodium acetate (pH 5.2) and either 2 vols. of absolute ethanol or 0.8 vols. of absolute isopropanol. Spin down the precipitated DNA, wash with 70% ethanol and re-dissolve in a suitable volume of buffer.</p>

Problem: Tissue lysate clogs spin column	
<u>Question / Problem</u>	<u>Help and comments</u>
Tissue lysate clogs spin column upon centrifugation and subsequent DNA yield is low.	<p>Too short incubation times with buffer T1/proteinase K may lead to insufficient lysis, causing clogging of the spin column and subsequently low DNA yield and quality.</p> <p>Time to reach complete solubilization of the tissue sample varies significantly between tissue types. If necessary, extend incubation time or increase the amount of proteinase K two-fold to obtain complete lysis.</p> <p>Some parts (e.g. hair and bones from mouse tails) will not dissolve readily upon treatment with buffer T1/proteinase K. If there is still undissolved matter present after an overnight incubation at 56 °C, spin down solid particles for 5 min. at max. speed and process only the clear supernatant in the next step.</p> <p>If mixing with buffers T1 and later T2 was insufficient, remaining cellular matter may clog the spin column and subsequent DNA yields will be low. In such a case repeat the DNA purification with new samples.</p>
Viscosity of the lysate is high	<p>If the tissue sample contains much DNA, the lysate may become very viscous due to the release of quite high amounts of high-molecular weight DNA. Viscous lysates may clog the spin column.</p> <p>To reduce viscosity, DOUBLE amounts of buffer T1, T2 and absolute ethanol. Mix immediately and thoroughly.</p> <p>This may require multiple rounds of loading the respective spin column.</p>
Lysate still doesn't go through properly.	Extend centrifugation time for the lysate to 5 min at maximally possible centrifugal force.

Problem: Colored remainders on membrane or in eluate after washing	
<u>Question / Problem</u>	<u>Help and comments</u>
DNA-containing eluate and membrane appear colored	<p>A heavily stained tissue sample may leave colored remainders on the membrane after processing of the tissue lysate through the spin column. If such remainders aren't removed completely, they may appear in the eluate, thus causing inhibition of downstream applications.</p> <p>In such a case make sure to carry out the buffer TX wash.</p> <p>If necessary, repeat the TX wash and let buffer TX sit on the membrane for 5 min before centrifugation.</p>

	<p>Too short incubation times with buffer T1/proteinase K may lead to insufficient tissue lysis, causing clogging of the spin column by colored solid remainders, thus subsequently causing low DNA yield and quality.</p> <p>Time to reach complete solubilization of the tissue sample varies significantly between tissue types. If necessary, extend incubation time or increase the amount of proteinase K two-fold to obtain complete lysis.</p> <p>Some parts (e.g. hair and bones from mouse tails) will not dissolve readily upon treatment with buffer T1/proteinase K. If there is still undissolved matter present after an overnight incubation at 56 °C, spin down solid particles for 5 min. at max. speed and process only the clear supernatant in the next step.</p> <p>If mixing with buffers T1 and later T2 was insufficient, remaining cellular matter may clog the spin column and leave colored material on the membrane surface. This will lead to low DNA yield and quality. In such a case repeat the DNA purification with new samples.</p>
	<p>Addition of absolute ethanol after cell lysis is mandatory to decrease foam and achieve optimal DNA purity.</p> <p>Omitting the addition of absolute ethanol to the sample before loading onto the spin column may lead to a severe decrease of DNA purity as residual colored material may remain on the membrane and later appear in the eluate.</p>
	<p>Make sure, that wash buffers TX and T3 are reconstituted properly with <u>absolute ethanol</u> as outlined on the respective bottle's label.</p> <p>Also make sure, that wash buffers TX and T3 are used in the correct order (TX before T3).</p>
<p>Membrane still appears slightly stained, but eluate is clear.</p>	<p>Check the DNA content in the eluate spectrophotometrically. Do not only measure A260 and A280 values, but also the A320 nm value. For pure DNA the absorption at 320 nm is close to zero. Contaminated DNA, that still contains residual hemin, will show elevated 320 nm absorptions.</p> <p>If 320 nm absorption is low, the DNA prepared is likely to working well in subsequent applications.</p>

Problem: Low $A_{260/280}$ ratio	
<u>Question / Problem</u>	<u>Help and comments</u>
260/280 ratio is too low (< 1,70)	<p>Some tissue samples may be heavily stained. If any colored remainders will come through into the eluate, they will compromise 260/280 ratio by raising the baseline drastically. If only absorbances at 260 nm and 280 nm are determined, the 260/280 ratio will be low.</p> <p>Remove colored remainders with a buffer TX wash.</p> <p>If necessary, repeat the TX wash and let buffer TX sit on the membrane for 5 min before centrifugation.</p>
	<p>Too short incubation times with buffer T1/proteinase K may lead to insufficient tissue lysis, causing clogging of the spin column by solid remainders, thus subsequently causing a low 260/280 ratio and poor DNA yield and quality.</p> <p>Time to reach complete solubilization of the tissue sample varies significantly between tissue types. If necessary, extend incubation time or increase the amount of proteinase K two-fold to obtain complete lysis.</p> <p>Some parts (e.g. hair and bones from mouse tails) will not dissolve readily upon treatment with buffer T1/proteinase K. If there is still undissolved matter present after an overnight incubation at 56°C, spin down solid particles for 5 min. at max. speed and process only the clear supernatant in the next step.</p> <p>If mixing with buffers T1 and later T2 was insufficient, remaining cellular matter may clog the spin column and leave colored material on the membrane surface. This will lead to low DNA yield and quality. In such a case repeat the DNA purification with new samples.</p>
	<p>Addition of absolute ethanol after cell lysis is mandatory to decrease foam and achieve optimal DNA purity.</p> <p>Omitting the addition of absolute ethanol to the sample before loading onto the spin column may lead to a severe decrease of DNA purity as residual colored material may remain on the membrane and later appear in the eluate.</p>
	<p>Make sure, that wash buffers TX and T3 are reconstituted properly with <u>absolute ethanol</u> as outlined on the respective bottle's label.</p> <p>Also make sure, that wash buffers TX and T3 are used in the correct order (TX before T3).</p>

	<p>Check the DNA content in the eluate spectrophotometrically. Do not only measure A260 and A280 values, but also the A320 nm value. For pure DNA the absorption at 320 nm is close to zero. Contaminated DNA, that still contains colored remainders, will show elevated 320 nm absorptions.</p> <p>An elevated 320 nm background will cause low A260/A280 ratios.</p>
<p>Animal blood processed?</p>	<p>Red blood stain from certain animal species may be difficult to remove.</p> <p>For example animals, that are fed with nutrients containing high amounts of proteins and carbohydrates (e.g. domestic cattle), may have quite viscous blood, that is more problematic to process.</p> <p>Other species' hemoglobin can be more difficult to remove (e.g. mouse and monkey).</p> <p>Residual hemoglobin or hemin is in any case a potent inhibitor of downstream applications.</p> <p>In such cases perform extra washes with buffer TX. If the buffer volume of buffer TX provided in the kit isn't enough, extra buffer can be ordered under catalogue number TX-500.</p> <p>If extra washes with buffer TX don't lead to the desired result, increase the proteinase K digestion time.</p>

<p>Problem: RNA contamination (High A_{260/280} ratio)</p>	
<p><u>Question / Problem</u></p>	<p><u>Help and comments</u></p>
<p>Prepared DNA still contains residual RNA</p>	<p>If RNA-free DNA is required, include the optional RNase digestion step as outlined in the protocol. RNase A is provided with each JETQUICK kit for tissue DNA purification.</p> <p>Add RNase before buffer T2 is added to the tissue lysate.</p>

Problem: Poor performance of the DNA in subsequent applications	
<u>Question / Problem</u>	<u>Help and comments</u>
DNA doesn't work in subsequent applications.	<p>Check the DNA content in the eluate spectrophotometrically. Do not only measure A260 and A280 values, but also the A320 nm value. For pure DNA the absorption at 320 nm is close to zero. Contaminated DNA, that still contains colored remainders, will show elevated 320 nm absorptions.</p> <p>An elevated 320 nm background will cause low A260/A280 ratios.</p> <p>Elevated 320 nm values are caused by residual contaminants, often being potent inhibitors for many enzymes.</p>
Residual ethanol from buffer T3 in the eluate	<p>Ethanol is a potent inhibitor of enzymatic reactions.</p> <p>If the silica membrane still smells significantly of ethanol after the centrifugation to remove residual buffer T3 directly before elution, either extend the time for this centrifugation step or incubate the spin column for 5 – 10 min in an incubator at 70°C.</p>
Not enough DNA in eluate	Look up the sections „Low yield of DNA“ and „Low DNA concentration in the eluate“ for possible reasons.
Elution buffer used.	Some downstream applications are sensitive to EDTA. Therefore don't use TE buffer, but either the 10 mM Tris-HCl (pH 9,0) buffer provided with the kit or double-distilled water for elution.
Inhibitory substances co-purified	Look up the sections „Tissue lysate clogs spin column“, „Colored remainders on membrane or in eluate after washing“ and „Low A _{260/280} ratio“ for possible reasons.

Problem: Degraded DNA	
<u>Question / Problem</u>	<u>Help and comments</u>
Prepared DNA appears degraded on a gel.	<p>DNA came into contact with nucleases during purification.</p> <ol style="list-style-type: none"> Check lab components for nuclease contamination. Autoclave glass and plasticware from the lab to inactivate nucleases. Tissue sample was not sufficiently reduced to small pieces before mixing with buffer T1 and proteinase K, so that cellular DNases were not completely inactivated. Repeat purification with fresh samples and make sure, that they are thoroughly mixed with buffer T1 and GENOMED protease.

Tissue sample was too old and not stored at -70°C to -80°C .

Tissue sample was repeatedly frozen and thawed. Avoid repeated freezing/thawing of the starting material.