JETQUICK Blood & Cell Culture Kit
Extended Troubleshooting Guide

Contents:

Low yield of DNA
Low DNA concentration in the eluate
Blood lysate clogs spin column
Colored remainders on membrane or in eluate after washing
Low $A_{260/280}$ ratio
RNA contamination
Poor performance of DNA in subsequent applications
Degraded DNA
Suggestions for the treatment of clotted blood

Problem: Low yield of DNA	
Problem	Help and comments
DNA yield from blood sample is lower than expected.	Determine the number of DNA-containing white blood cells in the whole blood sample. Blood from a healthy donor contains 5 x 10^6 – 1 x 10^7 white blood cells per ml. As a rule of thumb, 5 – 10 µg of genomic DNA can be expected from 200 µl of whole blood. If the number of white blood cells in the blood sample is low, use higher blood volumes and concentrate the white blood cells with WBC buffer (see protocol for preparation of DNA from 1 ml whole blood). As an alternative, prepare buffy coat from the blood sample and use only the buffy coat for DNA preparation.
	Make sure, that the cells in the blood sample are lysed efficiently by treatment with GENOMED protease and buffer K1. Mixing with buffer K1 is best carried out by bump- or pulse-vortexing for 10 – 20 sec. It is essential, that blood and buffer K1 are mixed thoroughly to yield a homogeneous solution. If mixing with buffer K1 was insufficient, remaining blood cells may clog the spin column and subsequent DNA yields will be low. In such a case repeat the DNA purification with new samples.

	DON'T add Genomed protease directly to buffer K1, e.g. to prepare a „master mix“ for serving multiple assays. Genomed protease will denature upon direct contact with buffer K1.
	Pay attention to the correct incubation temperature (58°C for blood mini preps, 70°C for Midi and Maxi preps) and the correct incubation time (at least 10 min). Too short incubation times may lead to insufficient cell lysis, thus causing clogging of the spin column.
	Addition of absolute ethanol after cell lysis is mandatory to decrease foam and achieve optimal DNA purity.
	Make sure, that wash buffers KX and K2 are reconstituted properly with absolute ethanol as outlined on the respective bottle's label.
	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. After having pipetted elution buffer onto the silica membrane, incubate for 5 min at room temperature. Shorter incubation times may lead to a reduced DNA recovery.
	Elution buffer should have a slightly basic pH (8.0 – 9.0) for optimal elution efficiency. Using plain water with a pH being <7.0 may lead to a decreased DNA yield.
Viscosity of the lysate is high	If the blood sample contains many DNA-containing cells, the lysate may become viscous due to the release of quite high amounts of high-molecular weight DNA. To reduce viscosity, add DOUBLE amounts of buffer K1 and absolute ethanol to the sample. Mix immediately and thoroughly. This may require multiple rounds of loading the respective spin column.

Problem: Low DNA concentration in the eluate

Question / Problem	Help and comments
DNA concentration in the eluate is too low.	DNA yield depends on the number of DNA-containing white blood cells in the whole blood sample. If DNA yield is low, DNA concentration in the eluate will be low, too. If the number of white blood cells in the blood sample is low, use higher blood volumes and concentrate the white blood cells with WBC buffer (see protocol for preparation of DNA from 1 ml whole blood). As an alternative, prepare buffy coat from the blood sample and use only the buffy coat for DNA preparation.
	For samples where a low DNA yield is to be expected, elution volume can be reduced by 50 – 75%, e.g. DNA from a blood miniprep can be eluted with 50 – 100 µl of elution buffer instead of 200 µl as outlined in the standard protocol. Protocols for blood DNA Midi and Maxi preps are giving guidelines of the percentage of DNA recovery to be expected with different volumes of elution buffer.
	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.

Problem: Blood lysate clogs spin column

Question / Problem	Help and comments
Blood lysate clogs spin column upon centrifugation and subsequent DNA yield is too low.	Make sure, that the cells in the blood sample are lysed efficiently by treatment with GENOMED protease and buffer K1. DO NOT mix GENOMED protease directly with buffer K1. Mixing with buffer K1 is best carried out by bump- or pulse-vortexing for 10 – 20 sec. It is essential, that blood and buffer K1 are mixed thoroughly to yield a homogeneous solution. If mixing with buffer K1 was insufficient, remaining blood cells may clog the spin column and subsequent DNA yields will be low. In such a case repeat the DNA purification with new samples.

	Pay attention to the correct incubation temperature (58°C for blood mini preps, 70°C for Midi and Maxi preps) and the correct incubation time (at least 10 min). Too short incubation times may lead to insufficient cell lysis, thus causing clogging of the spin column.
Viscosity of the lysate is high	If the blood sample contains many DNA-containing cells, the lysate may become viscous due to the release of quite high amounts of high-molecular weight DNA. To reduce viscosity, add DOUBLE amounts of buffer K1 and absolute ethanol to the sample. Mix immediately and thoroughly. This may require multiple rounds of loading the respective spin column.
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

Question / Problem	Help and comments
DNA-containing eluate and membrane appear colored	‚Brownish' or ‚reddish' appearing remainders in the eluate are from residual hemin, that wasn't removed completely. Be sure, to have the buffer KX wash carried out. If necessary, repeat the KX wash and let buffer KX sit on the membrane for 5 min before centrifugation.
	Make sure, that the cells in the blood sample are lysed efficiently by treatment with GENOMED protease and buffer K1. DO NOT mix GENOMED protease directly with buffer K1. Mixing with buffer K1 is best carried out by bump- or pulse-vortexing for 10 – 20 sec. It is essential, that blood and buffer K1 are mixed thoroughly to yield a homogeneous solution. If mixing with buffer K1 was insufficient, remaining blood cells may clog the spin column and/or residual red blood stain (hemin) may remain on the membrane and later appear in the eluate. As residual hemin is a potent inhibitor of PCR, repeat the DNA purification with new samples.

	Pay attention to the correct incubation temperature (58°C for blood mini preps, 70°C for Midi and Maxi preps) and the correct incubation time (at least 10 min). Too short incubation times may lead to insufficient cell lysis, thus causing clogging of the spin column or residual red blood stain (hemin) remaining on the membrane, and later appearing in the eluate.
	Addition of absolute ethanol after cell lysis is mandatory to decrease foam and achieve optimal DNA purity. 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 red blood stain (hemin) may remain on the membrane and later appear in the eluate.
	Make sure, that wash buffers KX and K2 are reconstituted properly with <u>absolute ethanol</u> as outlined on the respective bottle's label. Also make sure, that wash buffers KX and K2 are used in the correct order (KX **before** K2).
Membrane still appears slightly stained, but eluate is clear.	Check the DNA content in the eluate spectrophotometrically. Do not only measure A260 and A280 values, <u>**but also the A320 nm value**</u>. 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. If 320 nm absorption is low, the DNA prepared is likely to working well in subsequent applications.

Problem: Low $A_{260/280}$ ratio

<u>Question / Problem</u>	<u>Help and comments</u>
260/280 ratio is too low (< 1,70)	‚Brownish' or ‚reddish' appearing remainders in the eluate will cause a low A260/A280 ratio. These remainders are from residual hemin, that wasn't removed completely. Be sure, to have the buffer KX wash carried out. If necessary, repeat the KX wash and let buffer KX sit on the membrane for 5 min before centrifugation.

	Make sure, that the cells in the blood sample are lysed efficiently by treatment with GENOMED protease and buffer K1. DO NOT mix GENOMED protease directly with buffer K1. Mixing with buffer K1 is best carried out by bump- or pulse-vortexing for 10 – 20 sec. It is essential, that blood and buffer K1 are mixed thoroughly to yield a homogeneous solution. If mixing with buffer K1 was insufficient, remaining blood cells may clog the spin column and/or residual red blood stain (hemin) may remain on the membrane and later appear in the eluate. In such a case A260/A280 ratio will be low. As residual hemin is a potent inhibitor of PCR, repeat the DNA purification with new samples.
	Pay attention to the correct incubation temperature (58°C for blood mini preps, 70°C for Midi and Maxi preps) and the correct incubation time (at least 10 min). Too short incubation times may lead to insufficient cell lysis, thus causing clogging of the spin column or residual red blood stain (hemin) remaining on the membrane, and later appearing in the eluate. In such a case A260/A280 ratio will be low.
	Addition of absolute ethanol after cell lysis is mandatory to decrease foam and achieve optimal DNA purity. 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 red blood stain (hemin) may remain on the membrane and later appear in the eluate. In such a case A260/A280 ratio will be low.
	Make sure, that wash buffers KX and K2 are reconstituted properly with absolute ethanol as outlined on the respective bottle's label. Also make sure, that wash buffers KX and K2 are used in the correct order (KX **before** K2).
	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. An elevated 320 nm background will cause low A260/A280 ratios.

Blood from which species was processed?	Red blood stain from certain animal species may be difficult to remove. 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. Other species‘ hemoglobin can be more difficult to remove (e.g. mouse and monkey). Residual hemoglobin or hemin is in any case a potent inhibitor of downstream applications. In such cases perform extra washes with buffer KX. If the buffer volume of buffer KX provided in the kit isn't enough, extra buffer can be ordered under catalogue number KX-500. If extra washes with buffer KX don't lead tot he desired result, use proteinase K instead of GENOMED protease and/or increase the protease digestion time.

Problem: RNA contamination

Question / Problem	Help and comments
My prepared DNA still contains residual RNA	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 blood DNA purification. Add RNase **before** buffer K1 is added to the blood sample.

Problem: Poor performance of the DNA in subsequent applications

Question / Problem	Help and comments
DNA doesn't work in subsequent applications.	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. An elevated 320 nm background will cause low A260/A280 ratios. Elevated 320 nm values are caused in most cases by residual hemoglobin or hemin, being potent inhibitors for many enzymes.

Blood from which species was processed?	Red blood stain from certain animal species may be difficult to remove. 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. Other species‘ hemoglobin can be more difficult to remove (e.g. from mouse and monkey). Residual hemoglobin or hemin is in any case a potent inhibitor of downstream applications. In such cases one of the following suggestions may help: a) Use less blood. Try to decrease the amount of blood processed. b) Perform extra washes with buffer KX. If the buffer volume of buffer KX provided in the kit isn't enough, extra buffer can be ordered under catalogue number KX-500. c) If extra washes with buffer KX don't lead to the desired result, use proteinase K instead of GENOMED protease and/or increase the digestion time for the protease.
Residual ethanol from buffer K2 in the eluate	Ethanol is a potent inhibitor of enzymatic reactions. If the silica membrane still smells significantly of ethanol after the centrifugation to remove residual buffer K2 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.
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 „Blood 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

Question / Problem	Help and comments
Prepared DNA appears degraded on a gel.	DNA came into contact with nucleases during purification. a) Check lab components for nuclease contamination. Autoclave glass and plasticware from the lab to inactivate nucleases. b) Blood or cell culture sample was not sufficiently mixed with buffer K1, so that cellular DNases were not completely inactivated. Repeat purification with fresh samples and make sure, that they are thoroughly mixed with buffer K1 and GENOMED protease.
How old was the blood sample?	Whole blood specimen can be stored at 4°C for up to 5 days. If DNA cannot be purified within 5 days of collection, freeze aliquoted blood at –70°C to –80°C for future purification. Leaving blood at 4°C for an extended time-period may lead to DNA degradation.

Problem: Suggestions for the treatment of clotted blood

Question / Problem	Help and comments
Blood sample contains clots.	Clotted blood provides a real challenge for the JETQUICK system as blood clots may be recalcitrant against dissolution. The following suggestions may be helpful: a) Mince or mechanically disrupt the blood clot with a suitable pestle fitting into the reaction tube or another suitable device (e.g. a pasteur pipet). b) Per 200 mg of blood clot use 200 µl buffer K1 and 20 µl of a **proteinase K** solution (20 mg/ml) for proteolytic digestion. Incubate at 58°C for an extended time period (30 – 60 min), until all clotted material has dissolved. Proteinase K has a stronger proteolytic activity as GENOMED protease, so that it is the enzyme of choice for harder-to-lyse matters. c) If extended proteinase K treatment doesn't lead to the desired results, resuspend 200 mg of mechanically disrupted blood clot in 1 ml of WBC buffer (10 mM Tris-HCl [pH 7.6] / 5 mM $MgCl_2$ / 320 mM sucrose / 1% [w/v] Triton X-100). Incubate for 5 min, spin down the solid matter and remove the supernatant. Repeat the washes with WBC buffer until the pellet appears either white or only slightly colored.