

# JETQUICK MAXIPREP protocol for the purification of DNA from up to 20 ml of whole blood

**Reconstitute buffers KX and K2 with absolute ethanol as stated on the bottle's label!**

**Prepare GENOMED protease and RNase A as stated on the respective labels! Store the dissolved enzymes in single-use aliquots at -20°C. Avoid multiple freezing/thawing once the enzymes are dissolved in water.**

**All centrifugation steps in this protocol must be carried out in a swing-out rotor at 2,000 – 5,000 x g.**

**WBC buffer:** 10 mM Tris-HCl (pH 7,5),  
5 mM MgCl<sub>2</sub>,  
320 mM sucrose,  
1% Triton X-100.

- 1.) Pipette **up to 20 ml of whole blood** into a suitable 50 ml reaction tube (i.e. Greiner, Falcon®).
- 2.) Add **an identical amount** of WBC buffer to the blood sample and mix **thoroughly** by **inverting** the tube several times. Spin for **2 min** at **5,000 x g** in a swing-out rotor and pour off the supernatant. Be careful not to disturb or discard the pellet of white blood cells (WBC) at the bottom of the tube. Place the tube upside-down with the opening onto a sheet of absorbant paper towel and let residual liquid drain from the cell pellet.  
*The supernatant must be fully clear and translucent, the cell pellet appears light red at this stage.*
- 3.) Resuspend the white blood cell pellet in **9.5 ml PBS, TBS or another standard saline buffer** (to be provided by the user) by pulse-vortexing.
- 4.) Add **500 µl GENOMED protease** (20 mg/ml) and **10 ml Buffer K1** to the suspended cells from step 3 and **mix very thoroughly** by pulse-vortexing of the tube (e.g. three times for 5 seconds each).

**Do NOT add GENOMED protease directly to buffer K1. First mix the suspended cells with the enzyme, mix, and then add buffer K1.**

**Buffer K1:** Contains guanidine hydrochloride and detergent. These substances are irritants. Use with proper precaution! Wear gloves and safety goggles!

**OPTIONAL:** If you want to prepare **RNA-free genomic DNA**, first mix the suspended white blood cells with **300 µl of RNase A solution** (20 mg/ml). Then add the GENOMED protease as described above, mix thoroughly, add buffer K1, mix thoroughly again and proceed as described.

- 5.) Incubate for **10 min at 70 °C**.

*Optimal incubation temperature for GENOMED protease is 58 °C. However, to reach an optimal incubation temperature in the whole sample as quick as possible, thus obtaining optimal protein degradation in a short 10 min-period of time, we recommend to incubate the sample at 70 °C. If an incubation at the optimal temperature of 58 °C is wanted, extend the incubation time to 20 min.*

- 6.) Add **10 ml** of **absolute ethanol (96 – 100%)** to the mixture and mix ***immediately and very thoroughly*** by pulse-vortexing in order to prevent any precipitation of nucleic acids due to too high local alcohol concentrations.

*Do not use other alcohols than ethanol, because other alcohols may cause inconsistent yields.*

*Processing of too many white blood cells may lead to a high DNA content in the sample, so that DNA is partially precipitated. If any precipitated matter appears at this step, co-transfer it into the Maxi-spin column in step 7.*

- 7.) Assemble a JETQUICK MAXI-spin column with a 50 ml Receiver tube. Apply 15 ml of the sample from step 6 into the JETQUICK MAXI-spin column without moistening the rim of the column.

Close the tube with a cap, but don't screw the cap on too tight in order to allow ventilation during centrifugation.

Centrifuge the sample for **3 min** at **2,000 x g** in a swing-out rotor through the layer of silica membrane.

Discard the flowthrough. Then load the remainder of the lysate into the Maxi-Spin column and repeat the centrifugation as described.

*IMPORTANT NOTE 1: Centrifugation at lower speed will lead to an improved binding of the DNA to the membrane, thus achieving higher DNA yields!*

*IMPORTANT NOTE 2: If not all of the liquid has passed through the membrane, or if the surface of the membrane still appears significantly stained after the second portion of the lysate has been processed, re-centrifuge the spin column for another 1-2 min at 5,000 x g.*

- 8.) Discard the flowthrough. Apply **10 ml buffer KX (reconstituted with ethanol)** into the JETQUICK MAXI-spin column and centrifuge for **2 min** at **5,000 x g**.

- 9.) Discard the flowthrough and re-assemble the MAXI-spin column with the receiver tube. Apply **10 ml buffer K2 (reconstituted with ethanol)** into the JETQUICK MAXI-spin column and centrifuge for **2 min** at **5,000 x g**.

- 10.) Discard the flowthrough, re-assemble the MAXI-spin column and the receiver tube and centrifuge the empty tube again for **10 min** at **5,000 x g** in order to clear the silica membrane from residual liquid.

*If the maximally possible centrifugal force is  $\leq 4000 \times g$ , either an elongation of the centrifugation to up to 15 min, or an incubation of the JETQUICK Maxi column for 10 min at 70 °C in an incubator to evaporate residual ethanol is recommended. Residual ethanol in the eluate may cause inhibition of downstream enzymatic applications.*

- 11.) Insert the JETQUICK MAXI-spin column into a new, sterile 50 ml reaction tube (not provided with the kit) and elute the DNA from the membrane with **1 ml** of **10 mM Tris-HCl buffer (pH 9.0)**, TE buffer or simply water.

**The elution buffer should be used prewarmed to 70°C in order to achieve optimal DNA yields.**

Incubate the spin column for **5 min** at **room temperature** after application of the elution buffer and centrifuge subsequently for **2 min** at **5,000 x g**. The eluate now contains the pure DNA that can be further processed immediately.

**IMPORTANT:** Take your time and let the elution buffer re-dissolve the DNA from the membrane for 5 min. This leads to significantly improved DNA yields in comparison to shorter incubation times.

The absolute DNA yield can be increased by up to 15-20% by either doing a second elution step with another 1 ml of elution buffer or by carrying out the first elution step with 3 ml of elution buffer. The latter alternative, however, will lead to an eluate with a lower DNA concentration.

When carrying out 2 successive elution steps with equal amounts of elution buffer, we have found the following distribution of DNA in the two eluates:

<b>Elution volume</b>	<b>DNA in 1<sup>st</sup> eluate</b>	<b>DNA in 2<sup>nd</sup> eluate</b>
1 ml	80%	20%
2 ml	87%	13%
3 ml	92%	8%
4 ml	93%	7%

The DNA concentration in the first eluate can be increased by using this eluate for a second round of elution. Simply apply the eluate back into the Maxi-Spin column, let stand for another 5 min at room temperature and centrifuge as described.

We found the elution buffer that is provided with the kit (10 mM Tris-HCl [pH 9.0]) and other comparable elution buffers (e.g. bidistilled water, TE buffer: 10 mM Tris-HCl/0.1 mM EDTA [pH 8.0 – 8.5]) working equally well.