

JETQUICK protocol for the purification of DNA from whole blood, serum, plasma or other body fluids

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.

The JETQUICK Blood & Cell Culture Kit 50 (Cat. No. 440 050) is designed for 50 preparations, the JETQUICK Blood & Cell Culture Kit 250 (Cat. No. 440 250) for 250 preparations from 200 µl of whole blood.

1.) Pipette into a 1.5 ml or 2.0 ml reaction tube (i.e. Eppendorf) **up to 200 µl** of whole blood, serum, plasma, buffy coat oder another body fluid.

2.) Add **20 µl GENOMED protease** (20 mg/ml) and **200 µl Buffer K1** to this sample and **mix very thoroughly** either by vortexing or by inverting of the tube.

Do NOT add GENOMED protease directly to buffer K1. First mix the blood sample with the enzyme, mix, then add buffer K1.

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

OPTIONAL: If one wants to prepare RNA-free genomic DNA, add **10 µl of RNase A solution** (20 mg/ml) **prior** to the addition of buffer K1.

3.) Incubate for **10 min at 58°C**.

4.) Add **200 µl of absolute ethanol** to the mixture and mix **immediately and very thoroughly** 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.

5.) Assemble a JETQUICK micro-spin column with a 2 ml receiver tube. Apply the sample from step 4 into the JETQUICK micro-spin column without moistening the rim of the micro-spin cup and centrifuge the sample for **1 min at 10.000 x g** (approximately 10.600 rpm) through the silica membrane.

6.) Discard the flowthrough, re-assemble the Micro-Spin unit with the receiver tube and wash the JETQUICK column by applying **500 µl buffer KX reconstituted with ethanol** and centrifuging for **1 min at 10.000 x g** (approximately 10.600 rpm).

7.) Discard the flowthrough, re-assemble the Micro-Spin unit with the receiver tube and wash the JETQUICK column by applying **500 µl buffer K2 reconstituted with ethanol** and centrifuging for **1 min at 10.000 x g** (approximately 10.600 rpm).

- 8.) Discard the flowthrough, re-assemble the micro-spin and the receiver tube and centrifuge the empty tube again for **1 min at full speed (~13,000 rpm)** in order to clear the silica membrane from residual liquid.
- 9.) Insert the JETQUICK micro-spin tube into a new, sterile 1.5 ml reaction tube and elute the DNA from the membrane with **200 µl of 10 mM Tris-HCl buffer (pH 8,5)** or simply water.

The elution buffer should be used prewarmed to 70 °C and pipetted directly onto the center of the silica membrane. Take care that the whole membrane comes into contact with the elution buffer.

*Incubate the spin column for **2 min at room temperature** after application of the elution buffer and centrifuge subsequently for **2 min at 10.000 x g** (approximately 10.600 rpm). The eluate now contains the pure DNA that can be further processed immediately.*

Yield can be increased by up to 15% doing a second elution step with the same 200 µl of DNA-containing eluate. Preheat the 200 µl of eluate to 70 °C, fill it into the micro-spin device and proceed as described before.

*If lower DNA yields (< 3 µg) are to be expected, the volume of the elution buffer can be reduced to **100 µl** in order to obtain a higher DNA concentration in the eluate.*

*For expected DNA yields that are even < 1 µg, one can consider the reduction of the elution buffer volume to **50 µl**. However, any reduction of the elution buffer volume will lead to a somewhat reduced overall DNA yield of approximately 10-15%.*