

JETQUICK protocol for the purification of DNA from dried blood spots (November 2005)

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

Prepare proteinase K as stated on the respective label! Store the dissolved enzyme in single-use aliquots at -20°C. Avoid multiple freezing/thawing once the enzyme is dissolved in water.

This protocol is suitable for blood, both untreated and treated with anticoagulants, which has been spotted and dried on filter paper (e.g. Schleicher & Schuell 903 or comparable brand from other manufacturers). Prepare a 85°C water bath for use in step 2 and a 58°C water bath for use in steps 3 and 5. All centrifugation steps are carried out at room temperature.

- 1.) Place 3-6 punched-out circles from a dried blood spot into a 1.5 ml microfuge tube and add **200 µl of buffer T1** (available with the JETQUICK 'Tissue' kit or separately under catalogue number 'T1-200').
Use a 3 mm (1/8") single-hole paper puncher to cut out the punches from the dried blood spot.
- 2.) Incubate at 85°C for 10 min. Briefly centrifuge to remove drops from inside the lid.
- 3.) Add **20 µl Proteinase K** (20 mg/ml), mix by vortexing and incubate at 58°C for 1 h. Briefly centrifuge to remove drops from inside the lid.
- 4.) Add **200 µl Buffer K1** to this sample and **mix very thoroughly** either by vortexing or by inverting of the tube.
Buffer K1: Contains guanidine hydrochloride and detergent. These substances are irritants. Use with proper precaution! Wear gloves and safety goggles!
- 5.) Incubate for **10 min at 58°C**. Briefly centrifuge to remove drops from inside the lid.
- 6.) 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.
- 7.) Assemble a JETQUICK micro-spin column with a 2 ml receiver tube. Apply the sample from step 6 into the JETQUICK micro-spin column without moistening the rim of the micro-spin cup, thereby omitting the punched-out discs. Centrifuge the sample for **1 min at 10.000 x g** (approximately 10.600 rpm) through the silica membrane in the spin column.
- 8.) Discard the flowthrough, re-assemble the micro-spin unit with the receiver tube and wash the JETQUICK spin column by applying **500 µl reconstituted buffer KX** and centrifuging for **1 min at 10.000 x g** (approximately 10.600 rpm).

- 9.) Discard the flowthrough, re-assemble the micro-spin unit with the receiver tube and wash the JETQUICK column by applying **500 µl reconstituted buffer K2** and centrifuging for **1 min** at **10.000 x g** (approximately 10.600 rpm).
- 10.) Discard the flowthrough, re-assemble the micro-spin unit and the receiver tube and centrifuge the empty tube again for **1 min at max. speed (~13,000 rpm)** in order to clear the silica membrane from residual liquid.
- 11.) Insert the JETQUICK micro-spin tube into a new, sterile 1.5 ml reaction tube and elute the DNA from the membrane with **100 µl of 10 mM Tris-HCl buffer (pH 8,5)**, TE buffer 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. Then centrifuge 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.*

The yield can be increased somewhat by doing a second elution step with the same 100 µl of DNA-containing eluate. Preheat the 100 µl of eluate to 70 °C, re-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 **75 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 down to **50 µl**. However, any reduction of the elution buffer volume will lead to a somewhat reduced overall DNA yield of approximately 10-15%.*

Three punched-out circles with a diameter of 3 mm typically yield 75 – 150 ng of DNA from either untreated or anticoagulated blood. If the yield is not sufficient, use 6 circles per prep instead of 3.

The volume of the DNA eluate used in a PCR assay should not exceed 10%, i.e. for a 50 µl PCR assay use no more than 5 µl of eluate.