

# JETQUICK protocol for the purification of DNA from cultured cells

**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.**

1.) **A) Cells grown in suspension:** Harvest the cultures cells (maximally  $5 \times 10^6 - 1 \times 10^7$  cells with a normal set of chromosomes) in a 1.5 ml microfuge tube for 5 min at 300-350 x g. Remove the supernatant completely with a pipette. Do not disturb the cell pellet.

**B) Cells grown in monolayer.** Cells grown in monolayer can be detached from the culture flask by either trypsinization or using a cell scraper (e.g. a rubber policeman) according to established protocols.

**B1) Trypsinization:** Take off the medium supernatant from the monolayer and discard it. Wash the cell layer once with PBS. Aspirate the PBS and add trypsin solution (0.25% [w/v] trypsin / 0.2% EDTA / 0.9% NaCl) to the cells. When the cells have become detached from the wall of the dish or flask, collect the cells in medium and transfer an appropriate number (maximally  $5 \times 10^6 - 1 \times 10^7$  cells with a normal set of chromosomes) into a 1.5 ml microfuge tube. Spin down the cells for 5 min at 300-350 x g. Remove the supernatant completely with a pipette. Do not disturb the cell pellet.

**B2) Scraping off the cells:** Using a cell scraper, detach cells from the dish or flask. Transfer an appropriate number of cells (maximally  $5 \times 10^6 - 1 \times 10^7$  cells with a normal set of chromosomes) into a 1.5 ml microfuge tube. Spin down the cells for 5 min at 300-350 x g. Remove the supernatant completely with a pipette. Do not disturb the cell pellet.

2.) Resuspend the cell pellet in PBS, TBS or equivalent standard saline buffer (to be provided by the user) **to a final volume of 200 µl.**

3.) 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 suspended cells 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.

4.) Incubate for **10 min at 58°C.**

5.) 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.*

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

- 6.) Assemble a JETQUICK micro-spin column with a 2 ml receiver tube. Apply the sample from step 5 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.
- 7.) 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).
- 8.) 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).
- 9.) 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.
- 10.) 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. One should 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, 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 **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 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%.*