

JETQUICK MIDIPREP protocol for the purification of DNA from cultured cells, lymphocytes & bone marrow

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

**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 MIDI Kit 20 (Cat. no. 441 020) is designed for 20 preparations, the JETQUICK Blood & Cell Culture MIDI Kit 50 (Cat. no. 441 050) for 50 preparations from $7,5 \times 10^7 - 1 \times 10^8$ DNA-containing cells with a normal set of chromosomes.

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

1.) **A) Cells grown in suspension:** Harvest the cultures cells (maximally $7,5 \times 10^7 - 1 \times 10^8$ cells with a normal set of chromosomes) in a 15 ml centrifuge 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 $7,5 \times 10^7 - 1 \times 10^8$ cells with a normal set of chromosomes) into a 15 ml centrifuge 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 $7,5 \times 10^7 - 1 \times 10^8$ cells with a normal set of chromosomes) into a 15 ml centrifuge 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 3 ml.**

3.) To **3 ml cell suspension** from step 2 add **300 µl GENOMED protease** (20 mg/ml) and **3 ml Buffer K1** 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 resuspended 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 3 ml of cell suspension with **100 µl of RNase A solution** (20 mg/ml). Then add GENOMED protease as described above, mix thoroughly, add buffer K1, mix thoroughly again and proceed as described.

- 4.) 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.***

- 5.) Add **3 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.

- 6.) Assemble a JETQUICK MIDI-spin column with a 50 ml receiver tube. Apply the whole sample from step 5 into the JETQUICK MIDI-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 the 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.

IMPORTANT NOTE 1: A 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 processing of the lysate, re-centrifuge the spin column for another 2 min at 5,000 x g.

- 7.) Discard the flowthrough. Apply **10 ml buffer KX (reconstituted with ethanol)** into the JETQUICK MIDI-spin column and centrifuge for **2 min at 5,000 x g**.
- 8.) Discard the flowthrough and re-assemble the MIDI-spin column with the receiver tube. Apply **10 ml buffer K2 (reconstituted with ethanol)** into the JETQUICK MIDI-spin column and centrifuge for **2 min at 5,000 x g**.
- 9.) Discard the flowthrough, re-assemble the JETQUICK MIDI-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 $\leq 4,000 \times g$, either an elongation of the centrifugation to up to 15 min, or an incubation of the JETQUICK Midi-spin 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.

- 10.) Insert the JETQUICK MIDI-spin column into a new, sterile 50 ml reaction tube and elute the DNA from the membrane with **0.8 ml of 10 mM Tris-HCl buffer (pH 9.0) (provided)**, 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 8-10% by either doing a second elution step with another 0,8 ml of elution buffer or by carrying out the first elution step with 1 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 the amount of elution buffer as stated in the table below for the first elution and 1.0 ml for a second elution, we have found the following distribution of DNA in the two eluates:

Elution volume for FIRST eluate	DNA in 1st eluate	DNA in 2nd eluate (1.0 ml)
0.3 ml	41%	59%
0.5 ml	60%	40%
0.8 ml	75%	25%
1.0 ml	83%	17%

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 Midi-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.