

JETQUICK protocol for the purification of DNA from buccal swabs

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.

Swabs in general are the least invasive way of collecting DNA specimen from humans and animals for diagnostic purposes. In contrast to blood samples swabs have the additional benefit of minimizing the exposure to blood-borne pathogens.

The collection of swabs (buccal, nasal, pharyngeal, vaginal) are the method of choice when obtaining specimen for DNA testing. Swabs are easy to use and readily available. Cells collected on buccal swabs do not require special storage conditions and the DNA remains usable even after years of storage.

The JETQUICK Blood & Cell Culture Kit provides a fast and very efficient method for isolating genomic DNA from nearly all kind of swabs. The DNA isolated from the swabs is an ideal template for subsequent diagnostic PCR assays.

This protocol requires PBS, TBS or equivalent standard saline buffer for the resuspension of isolated cells. This buffer is not part of the kit and has to be provided by the respective user.

Due to an increased volume of buffer K1, that is required for the buccal swab protocol, fewer preparations can be carried out with the buffer volume provided with the standard kit. Additional buffer K1 (200 ml portions) can be purchased separately under catalogue number K1-200. Please inquire for this item at GENOMED or at your local distributor.

- 1.) Collect the buccal swab with a suitable tool. Nasal, pharyngeal or vaginal swabs can be collected in a similar way.

To collect a sample properly, make sure, that the donor abstains from consuming any foods or drinks at least 30 min before sample collection. To further reduce possible interference from unwanted components, the donor optionally can rinse his mouth twice with water immediately before sample collection.

Suitable tools for the collection of buccal swabs are:

- T-Swab Kit (Isohelix),
- DACRON® swabs (Fitzco),
- C.E.P. Omni swabs (Whatman),
- Cotton swabs (e.g. TriTech, Inc. or Puritan Hardwood Products).

- 2.) Collect the swab sample by rubbing the buccal swab brush firmly on the inside of the cheek, approximately 6-10 times on each side of the brush. Be sure to move the brush over the entire cheek.

*OPTIONAL: At this point you can dry the swab for **long-term storage**. Air-dry the swab for approximately 2 hours at room temperature. When completely dry, the swab can be stored in a suitable bag, that is usually delivered together with the swabs. Dried swabs can be stored for more than 1 year without affecting the DNA. To process dried swabs, simply transfer them into a capped 2 ml microcentrifuge tube and go ahead beginning with step 3 of this protocol.*

*IMPORTANT NOTE: Any swabs containing cellular material should **ONLY** be treated with gloves. Don't touch the swab with your finger tips or let it come into contact with your skin, as this may lead to contaminations and false-positive or false-negative results.*

3.) Place buccal swab into a capped 2 ml microcentrifuge tube (not provided with the kit). While C.E.P. Omni swabs can be ejected into the microcentrifuge tube by pressing the stem end towards the swab, the handle from other swabs can either be cut off by scissors or snapped off at the break point. The swab should fit entirely inside the tube allowing the cap to close.

4.) Add **600 µl of PBS, TBS or equivalent standard saline buffer** (to be provided by the user) to the swab. Mix briefly by vortexing.

5.) Add **20 µl GENOMED protease** (20 mg/ml) and **600 µl Buffer K1** to this sample and ***mix very thoroughly*** by vortexing 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.

6.) Incubate for **10 min at 58°C**.

7.) Add **600 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.

8.) Assemble a JETQUICK micro-spin column with a 2 ml receiver tube. Apply 700 µl of the sample from step 7 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.

Discard the flowthrough and repeat this loading and centrifugation step until all liquid from step 7 has been processed over the micro-spin column. Squeeze out residual liquid from the swab and discard the swab.

9.) 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).

- 10.) 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).
- 11.) 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.
- 12.) Insert the JETQUICK micro-spin tube into a new, sterile 1.5 ml reaction tube and elute the DNA from the membrane with **150 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 150 µl of DNA-containing eluate. Preheat the 150 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%.*