JETWELL 96-well Genomic DNA Purification from Cultured Cells (September 2005)

The JETWELL 96-well blood genomic DNA purification system can not only be used to prepare genomic DNA from whole blood or other body fluids, but also from lymphocytes, bone marrow or cultured cells.

For the long-term storage of GENOMED protease and RNase A we recommend dissolving the enzymes in water as stated on the accompanying label, divide it into single-use aliquots and store them at –20℃. Repeated freezing and thawing should be avoided.

Introduction

The JETWELL 96-well blood genomic DNA purification system is for the simultaneous purification of DNA from 96 samples originating from a wide range of clinical starting materials, among them bone marrow, lymphocytes and cultured cells that have a normal set of chromosomes.

The kit combines the selective binding properties of a unique silica membrane with a convenient high-throughput 96-well format. The DNA is highly pure and suitable for all enzymatic *in vitro* applications, like PCR, Southern blotting or restriction enzyme digestion. The DNA isolated with the kit has an average size of 30 – 50 kb, making it an ideal template for PCR amplification. DNA of this length denatures completely during thermal cycling and is amplified very efficiently.

The kit doesn’t use any toxic reagents like phenol, chloroform or ethidium bromide and yields DNA that is directly eluted in Tris or another low-salt buffer, thus being ready-to-use. All components should be at room temperature when used.

The Principle

The procedure is designed for cell culture samples with a size of 5×10^6 – 1×10^7 cells per well.

Depending on if the cultured cells have grown in suspension or as a monolayer, they are first harvested in a different way and resuspended in PBS, TBS or an equivalent saline buffer. The suspended cells are subsequently lysed with **GENOMED protease** in the presence of a chaotropic salt. After lysis has occured, absolute ethanol is added to ensure optimal binding to the membrane. This mixture is further processed over the silica membrane in a 96-well plate. During this passage, the genomic DNA will bind reversibly to the silica membrane. After two washes with different wash buffers to remove residual impurities, the genomic DNA is redissolved from the membrane with a low-salt elution buffer (provided with the kit).

The JETWELL 96-well plate procedure

The cell culture samples treated with lysis buffer, GENOMED protease and ethanol are loaded into the particular wells of the 96-well plate and processed over the silica membrane at the bottom of each well by centrifugation. During the processing over the membrane the DNA will bind reversibly to the silica.

After the removal of the unwanted components (i.e. proteins, metabolites, carbohydrates, lipids, salts) during the subsequent washing steps, the DNA is eluted in a low-salt buffer (10 mM Tris-Cl [pH 9.0], provided with the kit). The isolated DNA can directly be used for downstream applications.

When carrying out the procedure without the use of RNase, DNA and RNA will be purified simultaneously. RNA may inhibit some downstream enzymatic reactions. So, if RNA-free genomic DNA is required, the addition of RNase A to the cell samples is recommended (see protocol).

Protocol

The current protocol is for a centrifuge-driven 96-well procedure:

Preliminary steps & Important points

1.) **Dissolve the enzymes delivered with the kit (GENOMED protease, RNase A) in double-distilled or Milli-Q® (or equivalent grade) water as described on the instruction leaflet.**

2.) **Reconstitute buffers KX and K2 with absolute ethanol as described on the bottle's label. After having added the ethanol to the buffer concentrate, mix well by shaking.**

3.) Be careful when handling buffers K1 and KX as these buffers contain guanidine hydrochloride. This substance is an irritant. Wear gloves and goggles when handling these buffers.

4.) The volumes of buffers K1, KX and K2, as well as GENOMED protease and RNase provided with the kit are suitable for **200 µl whole blood samples**. The kit, however, is able to process larger samples of up to 600 µl.
If larger blood samples are to be processed, extra buffer and enzymes have to be purchased.

5.) If a precipitate is present in buffer K1, re-dissolve it by a short incubation at 50 – 55°C.

6.) All pipetting steps should be carried out with a suitable multichannel pipette.

7.) All steps, where vigorous mixing is required should be carried out by sealing the respective plate tightly with a suitable plastic mat (provided with the kit) and shaking vigorously up and down for at least 15 seconds, thereby holding the block with both hands. Shaking the block with only one hand will result in less efficient mixing in the wells on the side of the block held in the hand, with lower yields being obtained from those wells.

Suitable Centrifuges & Rotors

Centrifuge/Rotor	Centrifuge name	Centrifuge model	Rotor no.	Max. g-value	Max. plate height
Beckman-Coulter	TJ-25	Benchtop	S 5700	6,130	80 mm
Eppendorf	5810	Benchtop	A-2-DWP	2,250	89 mm
Eppendorf	5810R	Benchtop	A-2-DWP	2,250	89 mm
Heraeus Kendro	Multifuge 3	Benchtop	HIGHplate	5,350	85 mm
Heraeus Kendro	Multifuge 3-R	Benchtop	HIGHplate	5,650	85 mm
Hettich	Rotanta 460/460R	Benchtop	4620	5,858	86 mm
Hettich	Rotixa 50RS	Floor model	4282	2,695	86 mm
Jouan	BR4i / B4i	Benchtop	S-20	2,160	74 mm
Sigma	4K10	Benchtop	11144	2,991	70 mm
Tomy Tech Inc.	LX-130	Floor model	B-240-96D	3,010	70 mm

Centrifuge procedure

The centrifuge procedure is calling for a centrifuge with a suitable rotor capable of holding 96-well plates (see reference table).

During all centrifugation steps (except the elution step) the openings of the JETWELL 96-well plate can (but not need to) be sealed with adhesive air-permeative tape (not provided with the kit) to prevent cross-contamination. If such a tape sheet is used, air permeability is very important to avoid underpressure in the wells during centrifugation.

An adhesive tape should also be used when re-using plates containing unused wells. Label used wells of a JETWELL 96-well plate with a waterproof marker pen. Cover unused wells with tape and store the JETWELL 96-well plate in the bag in which it was supplied. Before starting the next run, remove the tape and cover the previously used wells with fresh tape.

1.) Mount the JETWELL 96-well plate onto the Waste Collection Plate. Make sure, that the JETWELL 96-well plate fits securely to the Waste Collection Plate.

2.) Harvest cultured cells:

A) Cells grown in suspension: Pipet cultures cells (maximally $5 \times 10^6 – 1 \times 10^7$ cells with a normal set of chromosomes per assay) into a well of a Lysate Preparation Plate (provided with the kit). After collecting all samples, centrifuge 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 5×10^6 – 1×10^7 cells with a normal set of chromosomes per assay) into a well of a Lysate Preparation Plate (provided with the kit). After having collected all samples, centrifuge 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 5×10^6 – 1×10^7 cells with a normal set of chromosomes) into a well of a Lysate Preparation Plate (provided with the kit). After having collected all samples, centrifuge for 5 min at 300-350 x g. Remove the supernatant completely with a pipette. Do not disturb the cell pellet.

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

4.) Pipet **20 µl of GENOMED protease solution (20 mg/ml)** to each cell sample in the wells of the Lysate Preparation Plate.
Make sure, that all pipet tips touch near the base of each well and the rims of the wells remain dry.

Add **200 µl of buffer K1** to each sample, taking care not to wet the rims of the wells. Seal the plate with the plastic mat provided and mix thoroughly by either shaking vigorously for at least 15 seconds or vortex-mixing on a suitable vortexer.

IMPORTANT NOTE: *As the sample cells regularly contain both DNA and RNA, both types of nucleic acids will be purified with this procedure. If RNA-free genomic DNA is to be prepared, add* ***10 µl of the RNAse stock solution (20 mg/ml; provided with the kit)*** *to each cell sample* ***before*** *adding buffer K1.*

For efficient lysis, it is essential that the samples and buffer K1 are mixed thoroughly to yield a homogeneous mixture. Hold the block with both hands and shake up and down vigorously. Alternatively, use a suitable vortexer.

Don't mix GENOMED protease directly with buffer K1 as this will denature the protein, thus causing an inactive enzyme.

5.) Incubate the block with the cell samples for **10 min at 58°C** either in an incubator or a water bath.
Placing a weight on top of the round-well block will prevent the lids popping off during incubation.

6.) Centrifuge briefly in a 96-well plate rotor at **3,000 x g** to collect all liquid at the bottom of each well.
Allow centrifuge to reach 3,000 x g, then stop the centrifuge.

7.) Remove the plastic mat and add **200 µl of absolute ethanol (96-100%)** to each well. Seal the wells with the plastic mat again and mix the contents by either shaking vigorously for at least 15 seconds or vortexing on a suitable vortexer.

8.) Centrifuge briefly in a 96-well plate rotor at **3,000 x g** to collect all liquid at the bottom of each well.
Allow centrifuge to reach 3,000 x g, then stop the centrifuge.

9.) Carefully apply the mixture from step 8 into the wells of the JETWELL 96-well plate mounted on the Waste Collection Plate from step 1.
Take care not to wet the rims of the wells to avoid aerosol formation during centrifugation.

10.) Centrifuge for **2 min at maximum speed**. Check if there is any liquid remaining in one of the wells. If there is any remaining liquid, centrifuge for another 2 min at maximum speed.
Discard the flowthrough into the sink and blot the Waste Collection Plate dry on a stack of absorbent paper towels. Re-assemble the JETWELL 96-well plate with the Waste Collection Plate.

11.) Add **500 µl of reconstituted buffer KX** to each well and centrifuge for **2 min** at maximum speed.
Discard the flowthrough into the sink and blot the Waste Collection Plate dry on a stack of absorbent paper towels. Re-assemble the JETWELL 96-well plate with the Waste Collection Plate.

12.) Perform another washing step with **900 µl of reconstituted buffer K2**.
After having discarded the flowthrough and reassembled the JETWELL 96-well plate with the Waste Collection Plate, centrifuge for another **5 min** at maximum speed to remove last traces of remaining ethanol.
If the wells still smell significantly of ethanol after this centrifugation step, either extend the last centrifugation step, or (recommended) incubate the JETWELL 96-well plate for 10 min at 70°C in an incubator.

13.) Assemble the JETWELL 96-well plate with a 96-well collection plate, suitable for elution volumes of 80 – 100 µl.
Apply **100 – 150 µl of 10 mM Tris-Cl (pH 8.5) buffer** (or water or TE buffer) **prewarmed to 70°C** directly to the center of each well of the JETWELL 96-well plate. Incubate for **5 min** at room temperature, then centrifuge for **2 min** with maximum speed.
If the recovered volumes per well are uneven after the 2 min centrifugation, centrifuge for another 5 min at maximum speed to collect the remaining eluate.

14.) Measure the DNA yield spectrophotometrically by measuring the absorptions at 260, 280 and 320 nm, using the 320 nm value as a correction factor. Alternatively, scan a UV spectrum of the sample in the range of 200 – 320 nm. Pure DNA has a A_{260}/A_{280} ratio of 1.7 – 1.9.