

JETWELL 96-well General DNA CleanUp system

Introduction

The JETWELL 96-well General DNA CleanUp system is for the simultaneous purification of 96 *in vitro* DNA assays, e.g. from enzymatic treatments with restriction enzymes or polymerases or from crude plasmid preparations. Each well has a DNA capacity of ≥ 20 μg . The DNA is highly pure and suitable for all enzymatic *in vitro* applications, like fluorescent sequencing, radioactive sequencing, restriction enzyme digestion, cloning procedures and microarray analysis.

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 JETWELL 96-well General DNA CleanUp system combines the convenience of multiwell technology with the selective binding properties of a proprietary silica membrane. The binding conditions provided with the kit are optimized for the efficient recovery of DNAs ranging in size from 80 bp to 20 kb. Restriction enzymes, DNA polymerases, other DNA-modifying enzymes, degraded RNA, oligomers with a length of up to 40 nucleotides, unincorporated dNTP's and salts are removed to an extent of $\geq 99.5\%$.

The JETWELL 96-well plate procedure

The DNA-containing assay to be purified is mixed with binding buffer, then 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 unwanted components (e.g. enzymes, bacterial proteins and metabolites, oligos, residual degraded RNA, dNTP's, salts) during the following washing step, the DNA is eluted in a low-salt buffer (10 mM Tris-Cl [pH 9.0], provided). The isolated DNA can directly be used for downstream applications.

Protocol

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

Preliminary steps

1.) Reconstitute **buffer M2** with **absolute ethanol** as described on the bottle's label.

- 2.) Be careful when handling **buffer M1** as this buffer contains guanidine hydrochloride. This substance is an irritant. Wear gloves and goggles when handling this buffer.

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).

- 1.) Mount the JETWELL 96-well plate onto a Waste Collection Plate (provided with the kit). Make sure, that the JETWELL 96-well plate fits securely.
- 2.) Transfer the DNA-containing assays with a suitable pipet into the Mixing Plate (provided with the kit) and add **4 volumes of buffer M1** to **1 volume of DNA assay**. Mix thoroughly (i.e. mix 400 µl buffer M1 with 100 µl of DNA-containing liquid) by pipetting up and down. Transfer the mix into the wells of the JETWELL 96-well plate.
- 3.) Centrifuge for **5 min at 300 x g**. 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.

- 4.) Add **900 µl of reconstituted buffer M2** 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 and 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 for another 10 minutes or incubate the JETWELL 96-well plate for 5-10 min at 60-70 °C in an incubator (recommended).
- 5.) Assemble the JETWELL 96-well plate with a 96-well Collection Plate (provided with the kit), suitable for elution volumes of 80 – 100 µl. Apply **80 µl of 10 mM Tris-Cl (pH 8.5) buffer** (or water or TE buffer) directly to the center of each well of the JETWELL 96-well plate. Incubate for 1 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. An application volume of 80 µl should yield an eluate volume of approximately 60-62 µl.*
- 6.) The DNA can be used directly for downstream applications. For storage cover the 96-well Collection Plate with the Sealing Mat provided with the kit.
- 7.) 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.