NZYDNA Clean-up 96 well plate

Catalogue numbers:

MB20001, 2 plates
MB20002, 4 x 2 plates

Description

NZYDNA Clean-up 96 well plate kit is designed for the fast and direct clean-up of DNA solutions or PCR reaction mixtures in the microtiter plate format. This kit can be used manually or fully automated using liquid handling systems. Addition of a chaotropic salt buffer (NP) will allow the plate silica-gel based membrane to selectively adsorb up to 15 µg of PCR DNA that, after a double washing step, is subsequently eluted in a highly purified form. PCR products or DNA may be eluted in elution buffer or highly pure water (pH>8.0). Complete removal of primers, primer-dimers, salts, nucleotides and proteins (e.g., polymerases, restriction enzymes, BSA) is efficiently achieved. Eluted PCR products and DNA are ready to use for several applications including sequencing, cloning or microarray analysis. The kit is designed for use with vacuum manifolds without the need for centrifugation steps enabling complete automation of the DNA preparations. In case vaccum systems do not available, NZYDNA Clean-up 96 well plate kit is also compatible with centrifugation procedures.

Storage conditions and reagents preparation

All kit components can be stored at room temperature (20-25 °C) and are stable till the expiry date. Add 100 mL of ethanol to each one of the Wash Buffer bottles. After addition of ethanol Wash Buffer is stable at room temperature (20-25 °C) for up to one year.

System Components

Component	2 x 96 preps
NP Buffer	50 mL
Wash Buffer (concentrate)	3 x 25 mL
Elution Buffer (does not contain EDTA)	40 mL
NZYDNA Clean-up 96 well plate (yellow rings)	2
Elution plate (V-bottom)	2
96-deep-well blocks* (1.2 mL)	2

* For centrifugation processing only.

Protocol for PCR or DNA clean-up in the plate format

This kit was developed to use under vacuum, which requires a vacuum manifold, or under centrifugation. NZYDNA Clean-up 96 well plate kit provides accessory plates (e.g. 96-deep-well blocks or elution plates). According to the equipment available, proceed with vacuum (see Section A) or centrifuge procedure (see Section B).

A. Vacuum procedure

All purification steps should be carried out at **room temperature**.

Check if ethanol was added to Wash Buffer before starting.

1. For DNA solutions or PCR reactions with volumes below 100 µL adjust to a final reaction volume of 100 µL by adding 10 mM Tris-HCl buffer, pH 7.0, or nuclease free water (pH 7.0 – 7.5). This step is critical because it is mandatory that the ration of NP Buffer: PCR reaction is 2:1.

2. Prepare the vacuum manifold according to the manufacturer instructions.

3. Dispense 200 µL of NP Buffer to the NZYDNA Clean-up 96 well plate (yellow rings). Column wise processing is recommended.

Note: In case a lower number of samples are to be processed cover the non-used wells with a self-adhering plastic foil to maintain sufficient vacuum.

4. Transfer PCR samples to the NZYDNA Clean-up 96 well plate and mix by pipetting up and down up to 5 times.

Optionally, you can adjust the volume of the PCR reaction (as described in step 1) to 100 µL in the PCR plate and then add 200 µL of NP binding buffer. After carefully mixing the reaction in the PCR plate transfer the entire volume (approximately 300 µL) to the NZYDNA Clean-up 96 well plate.

5. Apply vacuum (-0.2 to -0.4 bar, 1 min) to allow samples to pass through the columns while DNA binds to the silica membrane.

Note: Check if all samples have passed through the columns before proceeding to next step.

6. Add 900 µL of Wash Buffer (with ethanol added) to each well of the NZYDNA Clean-up 96 well plate. Apply vacuum (-0.2 to -0.4 bar, 1 min) to allow washing of the DNA bound to the silica membrane.

Note: Check if all Wash Buffer has passed through the columns before proceeding to next step.

7. Repeat the washing step with 900 µL of Wash Buffer as described above.

8. Remove any residual washing buffer from the NZYDNA Clean-up 96 well plate by gently tapping the outlets of the plate onto a soft tissue until no drops come out. Re-assemble the plate into the vacuum manifold and apply vacuum (-0.3 to -0.4 bar) for 10-15 min to dry the silica membrane completely. This step is absolutely required to remove traces of ethanol, which inhibits enzymatic reactions.

9. After finishing the drying step, insert the elution plate with V-bottom into the manifold to recover purified DNA. Add 75-150 µL of Elution Buffer or highly pure water (pH 8.5) to each well of the NZYDNA Clean-up 96 well plate (see Elution efficiencies section). Incubate for 1-3 min at room temperature (optionally), apply vacuum (-0.3 to -0.4 bar) for 1-2 min and collect the eluted DNA.

Notes:

- The Elution Buffer should be dispensed onto the centre of the silica membrane.

- Eluting the purified DNA in a centrifuge may be necessary when the purified DNA is required at higher concentrations. Using a centrifuge allows the dispensing volume to be reduced down to 50-75 µL. Centrifuge for 2-4 min in a microtiter plate centrifuge at 5,600 x g to collect the DNA.

10. Remove the elution plate from vacuum manifold and seal it with the provided adhesive cover foil for further storage.

B. Centrifugation procedure

All purification steps should be carried out at **room temperature**.

All centrifugations should be carried out at **room temperature** in a microtiter plate centrifuge which reaches accelerations of 5,600-6,000 x *g*. It is recommended to perform 2 × 96 DNA preparations at one time to allow that the rotor is balanced.

Check if ethanol was added to Wash Buffer before starting.

1. For DNA solutions or PCR reactions with volumes below 100 µL adjust to a final reaction volume of 100 µL by adding 10 mM Tris-HCl buffer, pH 7.0, or nuclease free water (pH 7.0 – 7.5). This step is critical because it is mandatory that the ration of NP Buffer: PCR reaction is 2:1.

2. Place NZYDNA Clean-up 96 well plate (yellow rings) on top of a new 96-deep-well block and dispense 200 µL of NP Binding Buffer to each well of the NZYDNA Clean-up 96 well plate. Column wise processing is recommended.

Note: In case a lower number of samples are to be processed cover the non-used wells with a self-adhering plastic foil to maintain sufficient vacuum.

3. Transfer PCR samples to the NZYDNA Clean-up 96 well plate and mix by pipetting up and down up to 5 times.

Optionally, you can adjust the volume of the PCR reaction (as described in step 1) to 100 µL in the PCR plate and then add 200 µL of NP binding buffer. After carefully mixing the reaction in the PCR plate transfer the entire volume (approximately 300 µL) to the NZYDNA Clean-up 96 well plate.

4. Place NZYDNA Clean-up 96 well plate with 96-deep-well block onto the centrifugation carrier and centrifuge at 5,600 × *g* for 2 min to allow samples to pass through the columns while DNA binds to the silica membrane. Discard the flow-through from 96-deep-well block.

Note: Check if all samples have passed through the columns before proceeding to next step.

5. Add 900 µL of Wash Buffer (with ethanol added) to each well of the NZYDNA Clean-up 96 well plate. Centrifuge at 5,600 ×*g* for 2 min to allow washing of the DNA bound to the silica membrane. Discard the flow-through from 96-deep-well block.

Note: Check if all Wash Buffer has passed through the columns before proceeding to next step.

6. Repeat the washing step with 900 µL of Wash Buffer as described above.

7. Discard the flow-through from the 96-deep-well block and remove any residual washing buffer from the NZYDNA Clean-up 96 well plate by gently tapping the outlets of the plate onto a soft tissue until no drops come out. Re-assemble the plate into 96-deep-well block. Centrifuge at 5,600 ×*g* for 10-15 min to dry the silica membrane completely. This step is absolutely required to remove traces of ethanol, which inhibits enzymatic reactions.

8. After finishing the drying step, place the NZYDNA Clean-up 96 well plate on new elution plate with V-bottom to recover purified DNA. Add 75-150 µL of Elution Buffer or highly pure water (pH 8.5) to each well of the NZYDNA Clean-up 96 well plate (see Elution efficiencies section). Incubate for 1-3 min at room temperature (optionally). Centrifuge at 5,600 ×*g* for 2-4 min and collect the eluted DNA.

Note: The Elution Buffer should be dispensed onto the centre of the silica membrane.

9. Remove the elution plate and seal it with the supplied adhesive cover foil for further storage.

Elution efficiencies

The efficiency of the DNA elution depends on the pH of the elution buffer. Elution is most effective at pH 8.0–8.5. When using nuclease-free water for elution, the pH value should be checked and, if necessary, adjusted to 8.0–8.5. Lower pH of the selected elution buffer may lead to lower recoveries. Yield of larger DNA fragments (> 5–10 kbp) can be increased by using pre-warmed (70 °C) elution buffer. An elution volume of 75–150 µL (125 µL preferably) of

Elution Buffer, as well as a 3–5 min incubation at room temperature (18–25 °C) of the elution buffer on the silica membrane are recommended. When using a vacuum manifold, the recovery elution volumes vary from 35 to 110 µL, when the volumes of dispensed Elution Buffer vary from 75 to 150 µL, respectively. This protocol is designed for optimal purification of DNA fragments between 65 bp to 10 kbp, with typical recoveries ranging between 75 to 95% of the initial DNA. Elution efficiencies of DNA fragments between 10 – 20 kbp are around 50-75%.

Quality control assay

All components of NZYDNA Clean-up 96 well plate kit are tested following the isolation protocol described above for the purification of high salt DNA solutions and PCR products.

V1901

Certificate of Analysis

Test	Result
Functional assay	Pass

Approved by:

Patrícia Ponte
Senior Manager, Quality Systems

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