§§ Charm Biotech

Just-a-Plate™ 96 PCR Purification and Normalization Kit

Store at room temperature

Product Contents and Storage

The components included with **Just-a-Plate** 96 PCR Purification and Normalization Kit are listed below. Upon receipt store all components at room temperature. Sufficient reagents are included to perform 2 X 96, or 10 X 96 DNA purifications and normalizations.

Product Catalogue #	JN-120-2	JN-120-10
Purification Scale	2 X 96	10 X 96
Binding Plate (BP5)	2 plate	10 plates
Binding Buffer (NB7)	4.4 ml	22 ml
Washing Buffer (WB2)	4.8 ml	24 ml
Elution Buffer (EB1)	9.0 ml	45 ml

Product Description

The Just-a-Plate™ 96 PCR Purification and Normalization Kit is designed and optimized for simple, fast, and high-throughput purification and normalization of PCR products. Up to 96 PCR reactions can be purified and normalized simultaneously in a 96-well microplate format. No spin-column, filter plate, silica membrane, or magnetic beads are needed for the clean-up and no vacuum or filtration steps are required in the process. Based on our Solid Surface Reversible Binding (SSRB) technology the Just-a-Plate system utilizes a 96-well plate with its surface coated with proprietary turbo-binders acting to selectively capture and efficiently bind DNA products from reaction mixtures. After performing PCR, DNA fragments will specifically interact with the turbo binders and bind to the surface of the wells in the presence of binding buffer. Primers, fluorescent dyes, nucleotides, and other contaminants will remain in solution. While using a fixed amount of turbo binder coated on the surface of the binding plate, the binding of the PCR products can be controlled in a certain amount by the coated capturing molecules. After washing the plate to remove unbound material, the purified and normalized DNA products can easily be eluted in 10 mM Tris buffer. The eluted products from each well are all in similar quantity, about 25 ng per well. This kit allows PCR product purification and normalization in a single step, eliminating the tedious manual normalization process after sample purification for sequencing sample preparation. The normalized and purified ready-to-use PCR products are ideal for use in automated next-generation sequencing reactions requiring the same amount of template inputs, offering superior data quality and read length.

Feature Highlights

Fast to perform: The whole process takes about one hour and but it only takes less than 10 minutes of hands-on time to purify and normalize 96 PCR samples.

Easy to handle: All procedures have been optimized with standard 96-well plates for ease-of-use. Elimination of multiple times of sample and buffer filtration in the sample binding and washing step associated with conventional membrane method makes whole procedure very easy to perform. No tedious manual normalization steps as DNA concentration determination, and sample dilution are required.

Reliable quality: Consistent well-to-well performance, reliable cross-contamination prevention procedure and unique solid-surface capture technology provide consistent yield at high DNA quality without any contamination.

Kit specifications: The kits are designed to purify and to normalize DNA fragment size of greater than 100 bps from PCR reaction with an input concentration range from 10 ng/µl to 200 ng/µl. The output from each well is 25 ng after normalization.

Additional Materials Needed

- 96 – 100 % ethanol
- Multi-channel pipettor, tips, and reservoirs
- Adhesive foil plate cover or adhesive plastic plate cover
- Optional: Swing-bucket centrifuge capable of spinning microplates, such as Eppendorf Centrifuge 5804, Beckman GS-6, or Sorvall 6600

- Optional: Thermocycler
- Optional: Vortex

General Precautions

- This kit is for research use only. All due care and attention should be exercised in the handling of the kits.
- Wear a laboratory coat, disposable gloves, and eye protection when handling reagents and plate. Avoid ingestion and inhalation of reagents. Avoid skin contact with reagents in the kit. In case of contact, wash thoroughly with water. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. MSDS information is available upon request.
- Always use proper aseptic techniques to avoid nuclease contamination when working with PCR and sequencing reactions and use only sterile, new pipette tips to prevent cross contamination.
- After elution of nucleic acid products from the wells, unused wells may be sealed with an adhesive foil cover and stored in the bag at room temperature for later DNA purification and normalization for up to 6 months.

Preparation before Starting

- To prepare working Washing Buffer (WB2), for JN-120-2 kit, add 19.2 ml of 100 % ethanol to the bottle and mix well. For JN-120-10, add 96 ml of 100% ethanol. (Mark bottle that ethanol has been added!) – Store at room temperature and use WB2 containing ethanol within six (6) months.
- After PCR, cooling the reaction to room temperature before purification.

Experimental Procedure

The protocol below is for the purification and normalization of DNA fragments from 20-µl PCR mixtures using the Just-a-Plate 96 PCR Purification and Normalization Sequencing Reaction Kit. The purification procedure may be scaled down by proportionately adjusting all reagents throughout the procedure. Please call our technical support for more information.

Binding of PCR Products to Binding Plate

1. After completion of the PCR and equilibration at room temperature, transfer 20 µl of each reaction mixture from the PCR plate to the Binding Plate (BP5) with a multi-channel pipettor.
2. Mix the Binding Buffer (NB7) thoroughly by swirling the bottle. Add 20 µl of Binding Buffer (NB7) to each sample well. Mix well with PCR products by pipetting liquid up and down 5 – 6 times. (Note: Avoid introducing bubbles while pipetting.)
3. Seal the Binding Plate (BP5) with an adhesive plate cover and incubate at room temperature for 30 – 60 minutes. (Note: The plate can be incubated overnight at room temperature if it fits your workflow.)
4. Remove the adhesive plate cover.
5. Remove the liquid from the Binding Plate. Choose one of the methods listed below to remove the liquid. (1) Decant the solution by quickly flipping the plate over a waste container and shaking briskly, then place the inverted plate on a stack of clean absorbent paper, such as Kimwipe® or paper towels, and tap the plate on the clean paper 3 – 4 times gently to remove as much liquid as possible; (2) Remove the solution by aspirating the solution from each well with a multi-channel pipettor. Be sure not to scrape the walls of the wells during aspiration as the products are bound to the walls. For consistency, we recommend to use a liquid handling robot, such as Biomek FXP from Beckman, EpMotion from Eppendorf or EVO Series from Tecan, for all pipetting work.

   (Note: Please see below for sequential binding and elution, please use (2) method listed above to transfer DNA solution to a new well. Since only about 50 ng of PCR products is captured and purified from the PCR reaction from this procedure. Majority of PCR products is still in the solution after capture. The remaining PCR products in the solution can be easily purified with our PCR purification kit (Cat No.JA-100-2). Please call us for more information.)

Washing Plate

1. Add 50 µl Wash Buffer (WB2) containing ethanol to each well. Mix the solution by pipetting up and down 1 – 2 times. Incubate the plate for 30 seconds at room temperature.
2. Remove the Wash Buffer from the Binding Plate using one of the methods suggested in step 5 of Binding of PCR Products to Binding Plate above.
3. Repeat steps 1 and 2 above once for a total of two washes.
4. After the final wash, blot the plate dry on a piece of clean absorbent paper and air-dry the plate in a lab hood for 5 –10 minutes to remove any residual liquid. (Alternatively, put plate in a thermocycler or an air incubator and air-dry the plate without cover at 65 º C for 2 – 4 minutes.)

Eluting Products

PCR amplicons attached to the wall of the Binding Plate can be analyzed directly in the well by sequencing using a 20 – 50 µl reaction volume without elution. If the DNA is to be eluted, use the procedure below

1. Add 20 - 40 µl of Elution Buffer (EB1) into each well of the Binding Plate. (If higher concentration (such as, ~ 5 ng/µl) is preferred, add 10 µl elution buffer.)

2. Seal the plate with a new adhesive plate cover and vortex the plate for 30 seconds. Centrifuge the plate at maximum speed briefly to collect all liquid at the bottom of the wells.
3. The eluted DNA fragments may be used immediately for downstream NGS application. Alternatively, the eluted products may be stored in the sealed plate at 4 ° C for short-term storage or at –20 ° C for long-term storage.

DNA Quantitation and Downstream Application

The expected yield of DNA amplicons is about 25~50 ng per well normalized. You can use the purified and normalized DNA sample directly for your next-generation sequence (NGS) reactions or other applications. However if determination of DNA yield is required, the quantity of purified DNA may be determined by fluorescent DNA assays. UV spectrophotometric measurement is not recommended since this method is inaccurate for low concentration DNA samples. The purified and normalized products are substantially free of contaminants such as salts, dNTP and primers, and can be used for next-generation sequencing analysis and other downstream applications in which high purity and normalized DNA templates are required.

Optional: Sequential Binding and Elution

If more than 50 ng of DNA amplicons are required, one way to improve the final yield is to do sequential binding and elution using the same DNA/binding buffer mixtures and elution solution. After the first binding of amplicons/binding solution mixtures to a well, transfer the same mixtures to another fresh well/plate for second binding at room temperature for 30 - 60 minutes. After washing the well, elute the DNA amplicons with the same elution buffer from the 1st well to obtain higher yield. Please call technical support for other options to increase the yield.

Troubleshooting

Problem	Cause	Solution
Poor Normalization	Inconsistent handling	Do not scratch the plate surface while pipetting Be sure to put pipette tip at the exact bottom center of each well while aspiration. Avoid generating bubbles while pipetting. If possible, use a fine-sharp small opening pipette tip for aspiration to reduce scrapping. Using automated liquid handling device is recommended to improve pipetting consistency.
Low yield of product	Insufficient DNA input DNA degradation	Make sure the input DNA concentration is ≥ 10 ng/μl. Be sure to follow lab guidelines to prevent DNase contamination.

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