

# PCR product purification with QIAquick 96 PCR Purification Kit and the VIAFLO 96 handheld electronic pipette

## Introduction

QIAquick 96 PCR Purification Kit is suitable for purifying up to 10 µg of material for downstream applications, such as sequencing, cloning, labeling and microarrays. The kit facilitates the removal of impurities like primers, unincorporated nucleotides, buffers, salts, mineral oils, agarose and enzymes. The vacuum-driven process is much faster than centrifugation, and gives high, reproducible yields. It is important to avoid cross-contamination in nucleic acid

purification, and QIAGEN's column design is optimized to limit carryover of contaminants. Although QIAquick 96 provides a high throughput solution, the elution, washing and binding steps are very laborious and time consuming if performed manually. With VIAFLO 96 handheld electronic pipette, the hands-on time is reduced, as samples and reagents can be transferred to all 96 wells at once. This enables rapid and efficient, high throughput PCR clean-up.

### Key benefits:

- VIAFLO 96 and VIAFLO 384 allow simultaneous pipetting of up to 96 or 384 wells, respectively, maximize throughput of PCR purification by allowing transfer samples and reagents in a single step.
- The z-heights can be predefined, choosing the optimal value to prevent accidental scratching of the well membrane for more consistent results.
- Custom programming of the PCR product clean-up steps allows pipetting parameters, such as aspiration or dispensing speeds, to be predefined. Prompt messages guide the user through the entire pipetting protocol, which is especially useful when several pre-wetting steps are included.
- The VIAFLO 96 or VIAFLO 384's hands-free automatic mode ensures that the PCR clean up protocols are performed in the same way each time, maximizing reproducibility.

## Overview: how to purify PCR products with VIAFLO 96

### Experimental set-up

This protocol describes how PCR products are purified using a VIAFLO 96 handheld electronic pipette with a two position stage and the QIAGEN QIAquick® 96 PCR Purification Kit. The following procedure is based on the kit manufacturer's protocol for purification of 96 samples (up to 10 µg PCR products).

A 96 channel pipetting head (50-1250 µl) is used together with 1250 µl SHORT, Low Retention, Sterile, Filter GripTips. Customized VIALINK programs are provided to perform the binding, washing and elution steps. Before starting, ethanol (96-100 %) should be added to the Buffer PE concentrate.



## Overview of the purification steps:

- Step 1:** Binding
- Step 2:** Washing
- Step 3:** Elution

The initial set-up of the QIAvac 96 Vacuum Manifold consists of a waste tray on top of a QIAvac base, followed by a QIAquick 96 well plate (pink) mounted on a QIAvac 96 top plate, as shown in **Figure 1**.

The QIAvac has to be attached to a vacuum source (house vacuum or vacuum pump) that generates negative pressure between 100 and 600 mbar.



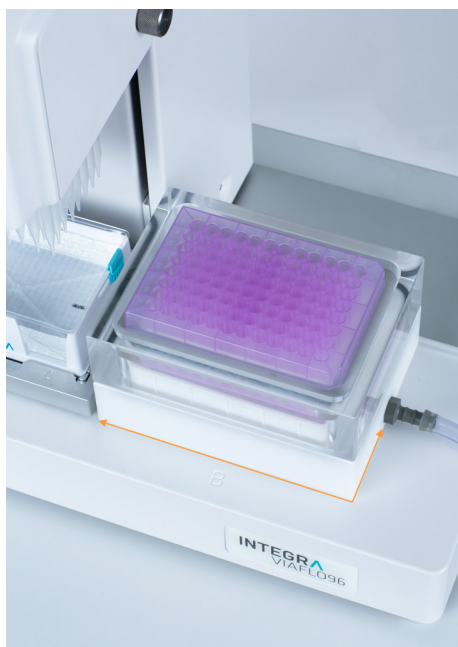
**Figure 1:** Initial set-up of the vacuum manifold.

## 1. Binding

**STEP:** Binding the DNA to the silica-gel membrane.

**HOW TO:** Load the 1250 µl SHORT, Low Retention, Sterile, Filter GripTips on the VIAFLO 96. Place a 150 ml Automation Friendly Reagent Reservoir in position A. The QIAvac 96 Vacuum Manifold should be placed on position B of the VIAFLO 96 in landscape orientation. No plateholder is needed on position B where the manifold is placed.

**Important:** The vacuum manifold should be aligned before each run (**Figure 2**).



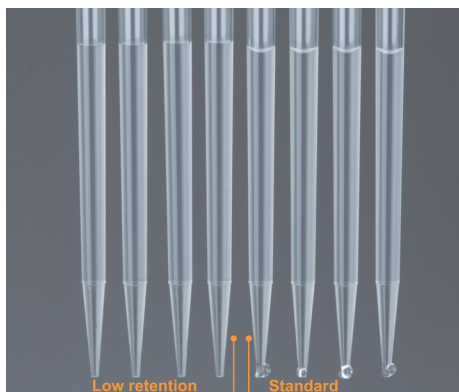
**Figure 2:** Alignment of the QIAvac 96 Vacuum Manifold.

Begin by launching the custom VIALINK program 'Qiaquick\_purification\_M'. The pipette will prompt the user to place Buffer PM on position A, then air is aspirated. This ensures that every single drop of the liquid can be dispensed later. The VIAFLO 96 will then guide the user through the two pre-wetting steps, starting with aspiration and dispensing 200  $\mu$ l of Buffer PM. After a second aspiration, the pipette will display the prompt 'Move the head out of buffer', before dispensing the final 200  $\mu$ l of Buffer PM. This is followed by a 20 second wait, giving the buffer residues time to flow down to the tip and be dispensed.

After pre-wetting, the pipette aspirates 75  $\mu$ l Buffer PM (three times the volume of the PCR product). The instrument then tells the user to remove the reservoir from position A, and replace it with the 96 well plate containing the 25  $\mu$ l of PCR products. After dispensing, and four mixing steps, the resulting mixture is transferred to the QIAquick plate wells in two steps. It is then time to switch on the vacuum source, as indicated by the pipette.

#### Tips:

- Pre-wetting the tips prior to pipetting prevents droplets and dripping when pipetting volatile liquids, such as isopropanol, which is one of the constituents in Buffer PM.
- Low Retention GripTips (**Figure 3**) are used for these pipetting steps to avoid dripping.



**Figure 3:** Low retention versus standard tips.

## 2. Washing

**STEP:** Two-step purification of the PCR product.

**HOW TO:** Eject the used tips and load new 1250 µl SHORT, Low Retention, Sterile, Filter GripTips on the VIAFLO 96. Place a new 300 ml automation friendly reagent reservoir in position A. The VIAFLO 96 will then prompt the user to pour Buffer PE into the reservoir, followed by a pre-wetting step, which is necessary since the buffer contains ethanol. After pre-wetting, the pipette will aspirate 900 µl of Buffer PE, and dispense it into QIAquick plate wells. The instrument will then notify the user that it is time to turn on the vacuum pump. With the pump turned on, another dose of the buffer is dispensed into the wells, followed by a 10 minute wait to dry the membrane and remove all residual ethanol.

**Important:** The final drying step is crucial to remove residual ethanol prior to elution. Residual ethanol in the elution buffer could inhibit downstream applications (e.g. PCR).

**Tip:**

- After this step, the manufacturer suggests tapping the plate on a stack of absorbent paper to ensure that all residual buffer is removed.

## 3. Elution

**STEP:** Elution of DNA from the silica-gel membrane.

**HOW TO:** When prompted, start by replacing the waste tray with the blue collection microtube rack provided, which contains 1.2 ml vessels (**Figure 4 a**). Load new 1250 µl SHORT, Low Retention, Sterile, Filter GripTips, and place a new 150 ml automation friendly reagent reservoir in position A. The instrument will then prompt the user to place Buffer EB into the reservoir, aspirate 80 µl, and dispense it into the QIAquick plate wells. After a 1 minute incubation, the pipette tells the user to switch on the vacuum source for 5 minutes.

**Tip:**

- The purified PCR product could also be eluted in a 96 well microplate. In this case, when replacing the waste tray, the 96 well microplate has to be placed on the empty blue collection tube rack (**Figure 4 b**).
- For increased DNA concentration, decrease the elution volume to 60 µl, as per QIAGEN's recommendations, in the VIALINK software.



**Figure 4:** Elution into a) provided collection microtubes or b) a 96 well microplate.

## Remark

### Vacuum manifold:

Alignment of the vacuum manifold is very important in this process. Adding marks on the deck helps to reposition the manifold whenever needed. To check the position of the well plate on top of the vacuum manifold, attach the tips manually to the pipette. The pipette tips should be in the middle of the wells. If not, adjust the position of the vacuum manifold on the deck.

### Automatic mode:

The VIAFLO 96 can also operate in hands-free automatic mode, allowing the user to have more walk-away time and less interaction, which is highly beneficial when using the instrument in a laminar flow cabinet. The customized automatic VIALINK program can be found in the download section.

## Conclusion

- The VIAFLO 96 electronic handheld pipette allows fast and simple liquid transfers for high throughput PCR product purification.
- Optimized pipette settings enable accurate sample and reagent transfer, without the tip touching and scratching the QIAquick membrane.
- The VIAFLO 96 electronic handheld pipette's compact design takes up minimal space and fits on any lab bench.
- The unique operating concept makes the VIAFLO 96 and VIAFLO 384 as easy to use as a conventional electronic pipette.
- The QIAvac 96 manifold is easily placed on the instrument and allows the processing of other kits using 96 well silica-membrane or filter plates.
- Another option for this application is the MINI 96, which is the most affordable 96 channel option on the market.

## Materials

Manufacturer	Part Number	Description	Link
INTEGRA	6031	VIAFLO 96 Base Unit	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA	6215	Spring loaded plate holder with slide function	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA	6104	96-Channel Pipetting Head 50 - 1250 µl	<a href="https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384">https://www.integra-biosciences.com/global/en/electronic-pipettes/viaflo-96-viaflo-384</a>
INTEGRA	6595	SHORT, Low Retention, Sterile, Filter GripTips for 96-channel pipetting head	<a href="https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide">https://www.integra-biosciences.com/global/en/pipette-tips/griptip-selector-guide</a>
INTEGRA	6338	150 ml automation friendly reservoir polypropylene	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
INTEGRA	6348	300 ml automation friendly reservoir polypropylene	<a href="https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs">https://www.integra-biosciences.com/global/en/reagent-reservoirs/automation-friendly-reagent-reservoirs</a>
QIAGEN	28181	QIAquick 96 PCR Purification Kit	<a href="https://www.qiagen.com/products/discovery-and-translational-research/dna-rna-purification/dna-purification/dna-clean-up/qiaquick-96-pcr-purification-kits/">https://www.qiagen.com/products/discovery-and-translational-research/dna-rna-purification/dna-purification/dna-clean-up/qiaquick-96-pcr-purification-kits/</a>
QIAGEN	19504	QIAvac 96 Vacuum Manifold	<a href="https://www.qiagen.com/products/discovery-and-translational-research/lab-essentials/vacuum-manifolds-and-accessories/qiavac-vacuum-systems/?catno=19504">https://www.qiagen.com/products/discovery-and-translational-research/lab-essentials/vacuum-manifolds-and-accessories/qiavac-vacuum-systems/?catno=19504</a>
VACUUBRAND	MZ 2 NT	Vacuum Pump	<a href="https://www.vacuubrand.com/en/page809.html">https://www.vacuubrand.com/en/page809.html</a>

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