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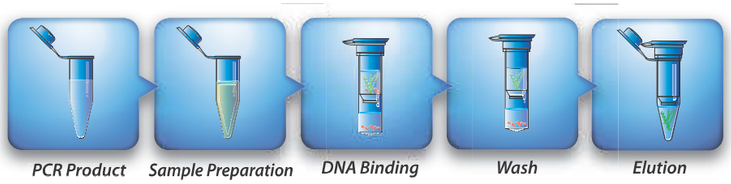
Store at room temperature 15°C~25°C



HiYield Plus™ PCR Purification Kit Protocol Book

Rapidly recover DNA fragments (70 bp - 20 kb) from PCR products

Cat. No. **QPP100 / QPP300**

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HiYield Plus™ PCR Purification Kit

HiYield Plus™ PCR Purification Kit

Store at room temperature 15°C~25°C

Cat. No. **QPP100**
100 mini preps / kit
QPP Buffer: 80 ml
pH Indicator: 360 µl
3M Sodium Acetate: 200 µl *
Wash Buffer (concentrated): 25 ml **
Elution Buffer: 6 ml
QPP Column: 100 pcs
2 ml Collection Tube: 100 pcs

Cat. No. **QPP300**
300 mini preps / kit
QPP Buffer: 240 ml
pH Indicator: 1 ml
3M Sodium Acetate: 200 µl *
Wash Buffer (concentrated): 75 ml **
Elution Buffer: 30 ml
QPP Column: 300 pcs
2 ml Collection Tube: 300 pcs

Sample: Up to 100 µl of PCR products
DNA fragment size: 70 bp-20 kb
Recovery: Up to 95%
Format: Spin column
Operation Time: 10 minutes
Elution Volume: 20-50 µl

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* pH Indicator allows easy determination of the optimal pH for DNA binding and easy observation of undissolved agarose gel. Efficient DNA adsorption requires a pH≤7.5, and the mixture of pH Indicator and QPP Buffer will appear yellow in this range. If the pH is >7.5, the binding mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the binding mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0).

** Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Description

HiYield Plus™ PCR Purification Kit is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from PCR or other enzymatic reactions within 10 minutes. This kit includes the pH Indicator. The optional pH Indicator can be added to QPP Buffer allowing easy determination of the optimal pH for DNA binding. Efficient DNA adsorption requires a pH≤7.5, and the pH Indicator in QPP Buffer appears yellow in this range. If the pH is >7.5, the mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0), which is also included in this kit. The color of the mixture allows easy visualization of optimal pH value, ensuring complete sample preparation and maximum yields. Typical recovery is up to 95% for PCR cleanup. The pH indicator, salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation and the purified DNA is ready for use in many downstream applications.

Features

Rapid purification of up 95% recovery of ready-to-use DNA within 10 minutes.
pH Indicator provides visual identification of optimal pH, ensuring maximum yields.
Suitable for wide range of DNA fragments (70 bp-20 kb).

Applications

Purified DNA is ready for direct use in restriction enzyme digestion, ligation, transformation, PCR, DNA sequencing, preparation of PCR probes for microarrays, DNA library screening and analysis.

Quality Control

The quality of HiYield Plus™ PCR Purification Kits are tested on a lot-to-lot basis. The efficiency of DNA recovery are tested by isolation of DNA fragments of various sizes from PCR products. The purified DNA is checked by agarose gel analysis.

Storage

HiYield Plus™ PCR Purification Kits should be stored dry at room temperature (15–25°C) for up to 12 months without showing any reduction in performance and quality.

Important Notes

Please read the entire notes before starting any of the protocol procedures.

PCR Sample Preparation

It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Buffer Notes

1. Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Most buffers contain irritants. Always wear a lab coat, disposable gloves, and protective goggles when handling these buffers.
3. The optional pH Indicator can be added to QPP Buffer allowing easy determination of the optimal pH for DNA binding. Efficient DNA adsorption requires a pH≤7.5, and the pH Indicator in QPP Buffer appears yellow in this range. If the pH is >7.5, the mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0), which is also included in this kit. The color of the mixture allows easy visualization of optimal pH value, ensuring complete sample preparation and maximum yields.

Centrifugation Notes

All centrifugation steps are carried out at 14,000 - 1,6000 x g in a conventional, table-top microcentrifuge at room temperature (15–25°C).

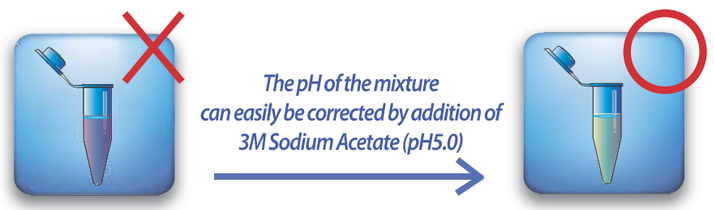
Elution Notes

1. Ensure that the elution buffer, TE or water is dispensed directly onto the center of the QPP Column in a 2 ml Collection Tube.
2. To increase DNA yield, use a higher elution buffer volume. To increase DNA concentration, use a lower elution buffer volume.
3. If plasmid DNA are larger than 5 kb, use pre-heated Elution Buffer (60~70°C).

4. Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. If plasmid DNA are larger than 5 kb, use pre-heated TE (60~70°C).
5. If using water for elution, ensure its pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the QPP Column matrix and is completely absorbed. If plasmid DNA are larger than 5 kb, use pre-heated water (60~70°C). DNA eluted in water should be stored at -20°C to avoid degradation.

How pH Indicator and 3M Sodium Acetate Work

The optional pH Indicator can be added to QPP Buffer allowing easy determination of the optimal pH for DNA binding. Efficient DNA adsorption requires a pH≤7.5, and the pH Indicator in QPP Buffer appears yellow in this range. If the pH is >7.5, the mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0), which is also included in this kit.



Protocol

Please read the entire important notes before starting any of the protocol procedures.

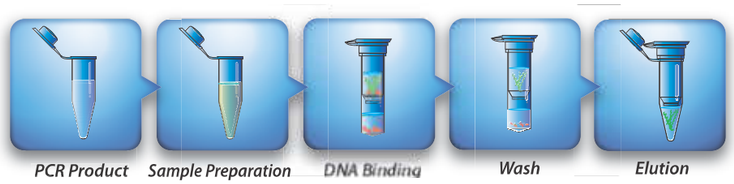
This protocol is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from PCR reactions using HiYield Plus™ PCR Purification Kit. Typical recovery is up to 95%. If the DNA fragment is larger than 5 kb, preheat the Elution Buffer, TE, or water to 70°C prior to the Elution Step.

Things to do before starting

1) Add absolute ethanol (see the bottle label for volume) to Wash Buffer and mix by shaking for a few seconds. Check the box on the bottle label showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2) It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

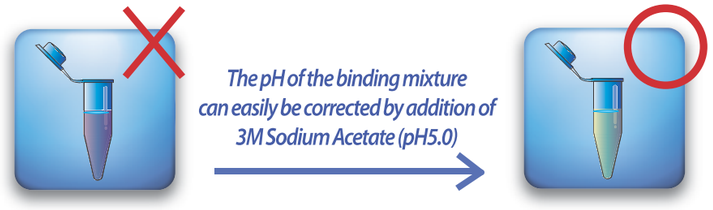
Additional Requirements

1) Absolute ethanol.
2) Sterile, DNase-free pipette tips and microcentrifuge tubes.



Sample Preparation

1. Transfer up to 100 µl of PCR reaction product to a 1.5 ml microcentrifuge tube. Add 5 volumes of QPP Buffer to 1 volume of the PCR sample and mix. It is not necessary to remove mineral oil or kerosene. For example, add 500 µl of QPP Buffer to 100 µl of PCR sample (not including oil).
2. If the color of the mixture has turned from yellow to purple, add 10 µl of 3M Sodium Acetate (pH5.0) and mix thoroughly. This will adjust pH and the color will return to yellow. Make sure the color of the mixture turns to yellow before proceed with next step.



DNA Binding

3. Place a QPP Column in a 2 ml Collection Tube. Transfer the sample mixture to the QPP Column.
4. Centrifuge at 14,000-16,000 x g for 30 seconds. Discard the flow-through then place the QPP Column back in the 2 ml Collection Tube.

Wash

5. Add 600 µl of Wash Buffer (absolute ethanol added) into the QPP Column and let stand for 1 minute.
6. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through.
7. Place the QPP Column back in the 2 ml Collection Tube.
8. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

Transfer the dried QPP Column to a new 1.5 ml microcentrifuge tube. Add 20-50 µl of Elution Buffer[1], TE[2] or water[3] into the CENTER of the column matrix. Let stand for at least 2 minutes to allow Elution Buffer, TE or water to be completely absorbed. Centrifuge at 14,000-16,000 x g for 2 minutes at room temperature to elute the purified DNA.

[1] If a higher DNA concentration is required, use 30 µl of Elution Buffer (10 mM Tris-HCl, pH8.5) then repeat the Elution step by adding the same 30 µl of Elution Buffer (which now contains the eluted DNA) to the center of the column matrix again. If maximum DNA yield is required, use 100 µl of Elution Buffer (DNA concentration will be diluted). Ensure that Elution Buffer is added into the center of the column matrix and is completely absorbed. If DNA fragment is larger than 5 kb, use pre-heated Elution Buffer (60~70°C).

[2] Using TE is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the QPP Column matrix and is completely absorbed.

[3] If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the QPP Column matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting

Problem	Possible Reasons / Solution
Low Yield	**Improper sample preparation.** 1. If using more than 100 µl of PCR products, separate it into multiple 1.5 ml microcentrifuge tubes. 2. Ensure the mixture of pH Indicator and QPP Buffer appears yellow before proceeding to DNA binding step. (If the the mixture turns violet. Violet color indicates DNA adsorption will be inefficient. The pH of the mixture can easily be corrected by addition of a small volume of 3M Sodium Acetate (pH5.0).
	Incomplete Wash Buffer preparation. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
	Incorrect DNA elution step. Ensure that Elution Buffer, TE or water is added into the CENTER of the Column matrix and is completely absorbed. If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.
Purified DNA does not perform well in downstream applications	**Primer dimer contamination in the final PCR elution product.** Gel purification should be performed if primer dimers are visible in the agarose gel following PCR reactions. Simply cut the PCR product from the gel and avoid the primer dimer. Using an additional 80% ethanol wash will reduce primer dimer contamination when performing PCR cleanup.

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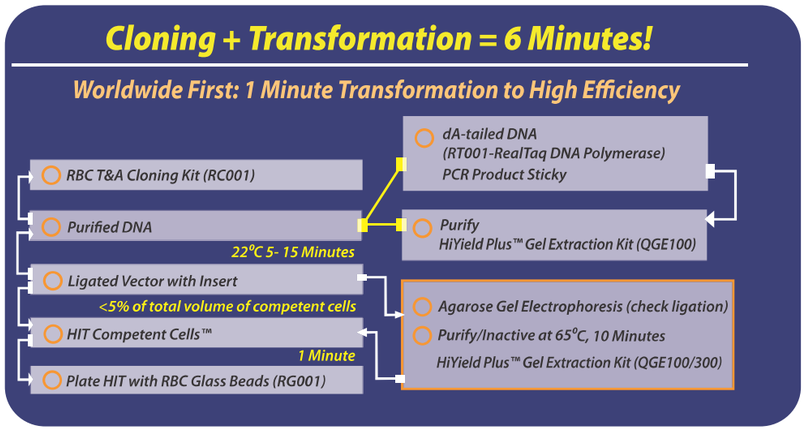
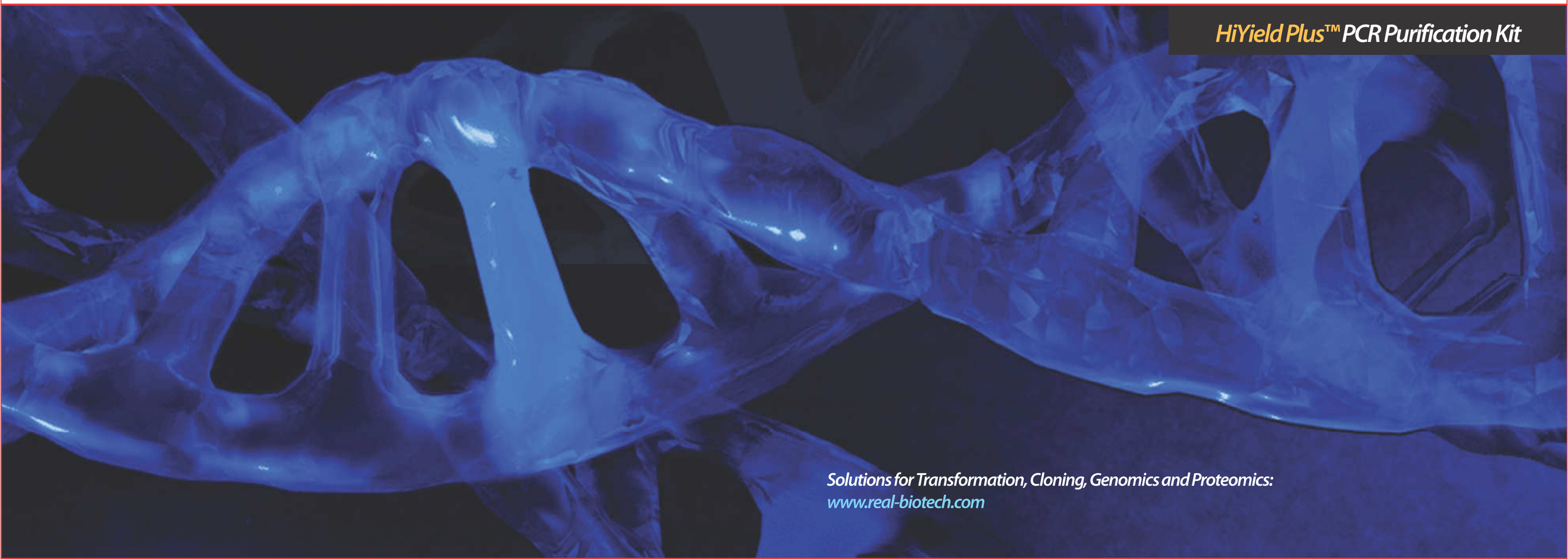


Ordering Information

Related products

	Cat. No.	Size	Items
Taq	RT001	S	RealTaq™ DNA Polymerase w/ dNTP, 500U/kit
	RTT01	L	RealTaq™ DNA Polymerase w/ dNTP, 2500U/kit
Cloning	RC001	S	RBC™ T&A Cloning Kit, 20 reactions/kit
	RC013	S	RBC™ T&A Cloning Vector, 20 reactions/kit
	RC011	S	RBC™ Rapid Ligation Kit, 100 reactions/kit
Gel Extraction PCR Cleanup	QGE100	S	HiYield Plus™ Gel Extraction Kit, 100 preps/ kit
	YDF100	S	HiYield™ Gel/PCR DNA Mini Kit, 100 preps/ kit
	QDF100	S	HiYield Plus™ Gel/PCR DNA Mini Kit, 100 preps/ kit
	YDS100	S	HiYield™ Gel/PCR Small DNA Mini Kit, 100 preps/ kit
	YDL100	S	HiYield™ Gel/PCR Large DNA Mini Kit, 100 preps/ kit
	YDM10	S	HiYield™ Gel/PCR DNA Maxi Kit, 10 preps/ kit
	YDF96B-2	S	HiYield™ 96-Well Gel/PCR DNA Extraction Kit, 2 preps/ kit
Transformation	RH617	S	HIT Competent Cells™ -DH5alpha Value 10^8, 100 µl x 10 vials, strain: DH5 alpha
	RH717	S	HIT Competent Cells™ -JM109 Value 10^8, 100 µl x 10 vials, strain: JM109
	RH117	S	HIT Competent Cells™ -Blue Value 10^8, 100 µl x 10 vials, strain: XL1-Blue
	RH217	S	HIT Competent Cells™ -BL21 Value 10^8, 100 µl x 10 vials, strain: BL21(DE3)
	RH517	S	HIT Competent Cells™ -DH10B Value 10^8, 100 µl x 10 vials, strain: DH10B
	RH317	S	HIT Competent Cells™ -GM2163 Value 10^8, 100 µl x 10 vials, strain: GM2163

HiYield Plus™ PCR Purification Kit



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