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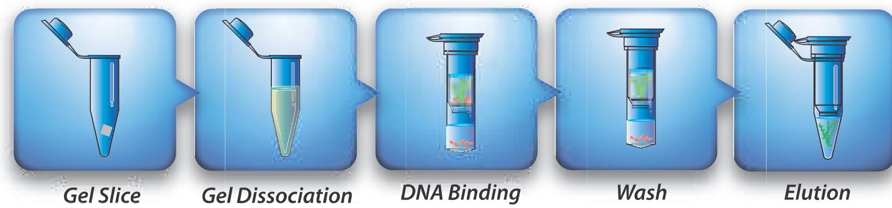
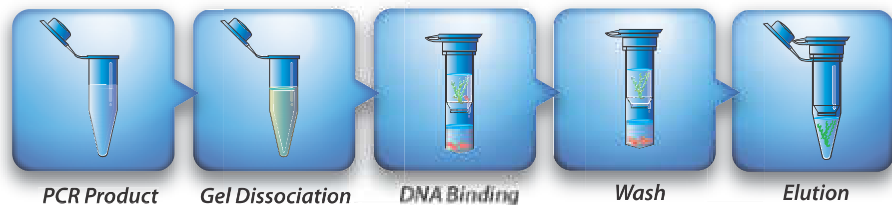
Workflow for Gel Extraction



Workflow for PCR Cleanup



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Real Genomics™

HiYield Plus™ Gel/PCR DNA Mini Kit Protocol Book

Optimized for DNA fragment between 70bp and 20 kb

Cat. No. **QDF100 / QDF300**

Real Genomics™

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HiYield Plus™ Gel/PCR DNA Mini Kit

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HiYield Plus™ Gel/PCR DNA Mini Kit

Cat. No. **QDF100**	Cat. No. **QDF300**
100 mini preps / kit	300 mini preps / kit
QDF Buffer: 80 ml	QDF Buffer: 240 ml
3M Sodium Acetate: 200 µl*	3M Sodium Acetate: 200 µl*
W1 Buffer : 45 ml	W1 Buffer : 130 ml
Wash Buffer (concentrated): 25 ml **	Wash Buffer (concentrated): 75 ml **
Elution Buffer: 6 ml	Elution Buffer: 30 ml
QDF Column: 100 pcs	QDF Column: 300 pcs
2 ml Collection Tube: 100 pcs	2 ml Collection Tube: 300 pcs

Sample: Up to 300 mg of agarose gel, up to 100 µl of PCR products
DNA fragment size: 70 bp-20 kb
Recovery: Up to 90% for gel extraction, up to 95% for PCR cleanup
Format: Spin column
Operation Time: 20 minutes for gel extraction, 10 minutes for PCR cleanup
Elution Volume: 20-50 µl

* QDF Buffer contains an integrated pH Indicator, allowing 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 pH Indicator in QDF 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™ Gel/PCR DNA Mini Kit is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from agarose gels, PCR or other enzymatic reactions within 20 minutes. QDF Buffer contains an integrated pH Indicator. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QDF 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 any unsolubilized agarose, ensuring complete solubilization and maximum yields. Typical recovery is up to 90% for gel extraction and 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 and ensures complete solubilization and maximum yields.
Unique dual-purpose design (gel extraction and PCR purification in one kit) makes this kit exceptional value.

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™ Gel/PCR DNA Mini 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 either aqueous solution or agarose gel. The purified DNA is checked by agarose gel analysis.

Storage

HiYield Plus™ Gel/PCR DNA Mini 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.

Gel Sample Preparation

1. Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
2. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.
3. Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

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. QDF Buffer contains an integrated pH Indicator, allowing 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 pH Indicator in QDF 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).

Centrifugation Notes

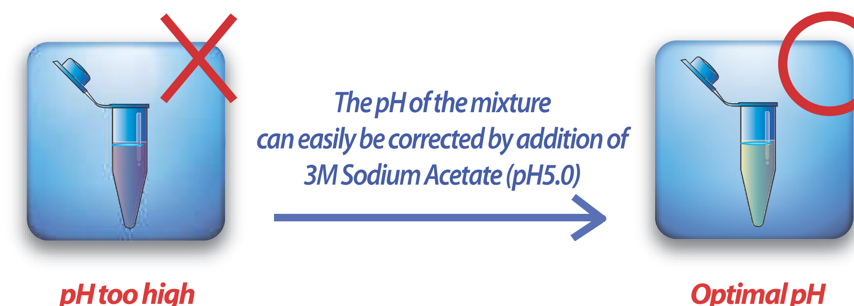
All centrifugation steps are carried out at 14,000 - 1,6000 x g in a conventional, table-top microcentrifuge at room temperature (15–25°C).

Elution Notes

1. Ensure that the elution buffer, TE or water is dispensed directly onto the center of the QDF 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 QDF 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

QDF Buffer contains an integrated pH Indicator. Efficient DNA adsorption requires a pH≤7.5,and the pH Indicator in QDF Buffer appears yellow in this range. If the pH is >7.5, the mixture will turn 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.



Gel Extraction Protocol

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

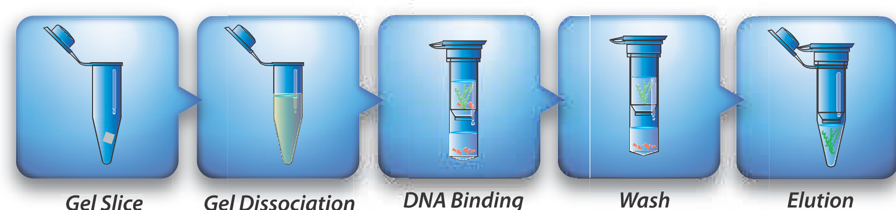
This protocol is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from agarose gels using HiYield Plus™ Gel/PCR DNA Mini Kit. Typical recovery is up to 90% for gel extraction. If the DNA fragment is larger than 5 kb, preheat the Elution Buffer, TE, or water to 70°C prior to the Elution 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) Record the weight of an empty 1.5 ml microcentrifuge tube for the gel dissociation step. Once the gel has been transferred to the tube, record the weight again. Subtract the empty tube weight from the total weight to determine the actual gel weight.
3) Perform gel purification when primer dimers are highly visible or add an additional 80% ethanol wash to avoid primer dimer contamination.

Additional Requirements

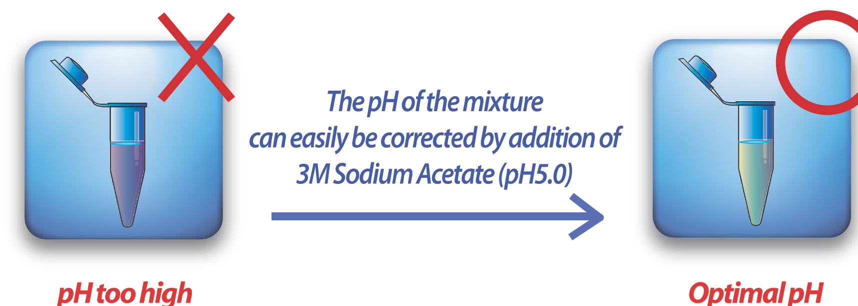
1) Absolute ethanol.
2) Sterile, DNase-free pipette tips and microcentrifuge tubes.



Gel Dissociation

1. Excise the DNA fragment from the agarose gel with a clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose. Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube.
2. Add 500 µl of QDF Buffer to the sample then mix by vortex. Incubate at 55-60°C for 10-15 minutes or until the gel slice is completely dissolved. During incubation, invert the tube every 2-3 minutes.
3. 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 is yellow then proceed with the next step.
4. Cool the dissolved sample mixture to room temperature.

Do not scale QDF Buffer if using less than 300 mg of gel slice. If using more than 300 mg of gel slice, separate it into multiple 1.5 ml microcentrifuge tubes.



DNA Binding

5. Place a QDF Column in a 2 ml Collection Tube. Transfer 800 µl of the sample mixture to the QDF Column.
6. Centrifuge at 14,000-16,000 x g for 30 seconds. Discard the flow-through then place the QDF Column back in the 2 ml Collection Tube.

If the sample mixture is more than 800 µl, repeat the DNA binding step.

Wash

Wash steps for DNA be used for direct sequencing, in vitro transcription, or microinjection

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

Standard wash steps

7. Add 400 µl of W1 Buffer into the QDF Column. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QDF Column back in the 2 ml Collection Tube.
8. Add 600 µl of Wash Buffer (absolute ethanol added) into the QDF Column and let stand for 1 minute. Centrifuge at 14,000-16,000 x g for 30 seconds then discard the flow-through. Place the QDF Column back in the 2 ml Collection Tube.
9. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.

DNA Elution

Transfer the dried QDF 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. Check elution notes listed in page 4 for more details.

[3] If using water for elution, check elution notes listed in page 4 for more details.

HiYield Plus™ Gel/PCR DNA Mini Kit

PCR Cleanup Protocol

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

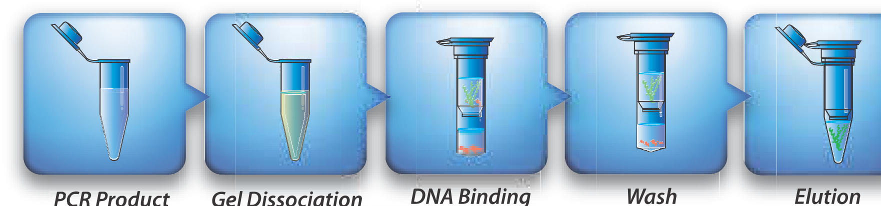
This protocol is designed to rapid recover or concentrate DNA fragments (70 bp-20 kb) from PCR reactions using HiYield Plus™ Gel/PCR DNA Mini Kit. Typical recovery is up to 95% for PCR cleanup. If the DNA fragment is larger than 5 kb, preheat the Elution Buffer, TE, or water to 70°C prior to the Elution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

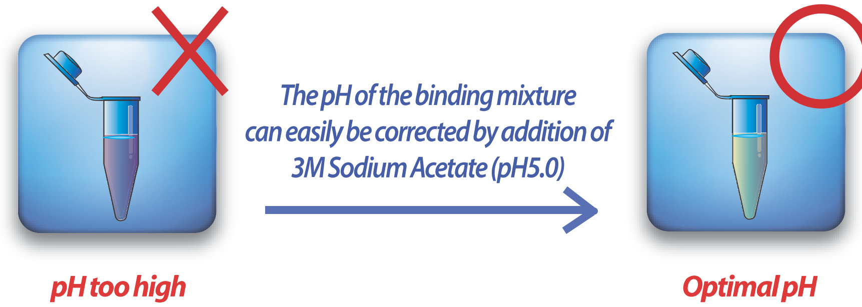
Additional Requirements

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

Wash

5. Add 600 µl of Wash Buffer (absolute ethanol added) into the QDF 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 QDF 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 QDF 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 QDF 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 QDF Column matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

HiYield Plus™ Gel/PCR DNA Mini Kit

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