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HiYield™ Gel / PCR Large DNA Mini Kit

Cat. No.:	YDL100	YDL300
Product Name:	HiYield™ Gel / PCR Large DNA Mini Kit	
Reactions:	100	300
Sample:	Up to 250 mg of either TAE or TBE agarose gel, up to 100 µl of PCR products	
DNA Size Range:	100 bp - 50 kb	
Recovery:	Up to 95% for DNA size of 100 bp - 10 kb, up to 80% for DNA size of 10 kb - 50 kb	
Format:	Modified silica bead	
Operation:	Centrifuge	
Operation Time:	30 minutes for gel extraction / 20 minutes for PCR cleanup	
Elution Volume:	10-20 µl	

Introduction

HiYield™ Gel/PCR Large DNA Mini Kit is designed to recover or concentrate DNA fragments (100 bp - 50 kb) from TAE or TBE agarose gel, PCR, or other enzymatic reactions by four simple steps within 20 minutes. DL Buffer has been optimized to dissolve either TAE or TBE gels. DNA can be extracted from TBE gels directly without TBE modifier.

DL Buffer contains an integrated pH Indicator. Efficient DNA adsorption requires a pH≤7.5, and the pH Indicator in DL 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 70%- 95% for DNA size between 100 bp - 10 kb and 60% - 80% for DNA size between 10 kb - 50 kb. The pH indicator, salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture without phenol extraction or alcohol precipitation. The purified DNA could be eluted in minimum volume (10 - 20 µl) and is ready for direct use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

Features

Rapid purification of up 95% recovery of ready-to-use DNA within 20 minutes.
2-in-1 design (gel extraction and PCR purification in one kit) makes this kit exceptional value.
Without phenol extraction or alcohol precipitation.

Applications

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

Contents

ITEM	YDL100	YDL300
DL Suspension Buffer	1.5 ml	4.5 ml
DL Buffer*	120 ml	360 ml
3M Sodium Acetate**	200 µl	200 µl
Wash Buffer (concentrated)***	25 ml	75 ml
Elution Buffer	6 ml	30 ml

*Routine purification from >2% agarose gel requires additional DL Buffer.

** After 50ºC incubation in the DNA binding step, check the mixture of DL Suspension Buffer and sample. If the color of the mixture becomes purple instead of yellow, the pH is too high. 3M Sodium Acetate (pH5.0) can then be added to adjust pH and the color will return to yellow.

*** 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.

Quality Control

The quality of HiYield™ Gel/PCR Large DNA Mini Kit is tested on a lot-to-lot basis. The efficiency of DNA recovery is tested by isolation of DNA fragments of various sizes from agarose gel. The purified DNA is checked by agarose gel analysis.

References

(1) Vogelstein, B., and Gillespie, D. (1979) Proc. Natl. Acad. Sci. USA 76, 615.

Gel Extraction Protocol

Please read the entire instruction manual prior to starting.

Caution: During operation, always wear a lab coat, disposable gloves, and protective goggles.

Things to Do before Starting:

1. 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.
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. Set heating block or water bath at 50ºC.

Additional Requirements: 1.5ml microcentrifuge tubes, absolute ethanol.

Step 1 **Gel** **Dissociation**	★Cut the TAE/TBE agarose gel slice containing relevant DNA fragments and remove any extra agarose to minimize the size of the gel slice. ★Transfer up to 250 mg of the gel slice to a 1.5 ml microcentrifuge tube. ★Add 600 µl of DL Buffer. NOTE: For >2% agarose gels, use 1.2 ml of DL Buffer.
Step 2 **DNA Binding**	★Resuspend DL Suspension Buffer by vortex for 30 seconds. Add 10 µl of DL Suspension Buffer for <2 µg of DNA to the gel sample then mix the tube thoroughly by vortex. NOTE: Add 20 µl of DL Suspension Buffer for 2-5 µg of DNA. ★Incubate at 50 ºC for 10 -15 minutes to ensure the gel slice has dissolved completely. During incubation, vortex the tube every 2-3 minutes. NOTE:If the color of the mixture has turned from yellow to purple, add 10 µl of Sodium Acetate (pH5.0) and mix thoroughly. This will adjust the pH and the color will return to yellow. Once the color has returned to yellow, incubate at room temperature for 5 minutes, mixing every 2 minutes. Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer then remove the supernatant using a pipette.
Step 3 **Wash**	★Add 500 µl of DL Buffer to the pelleted DL Suspension Buffer then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer then remove the supernatant with a pipette. ★Add 500 µl of Wash Buffer (make sure ethanol was added) to the pelleted DL Suspension Buffer then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer. Remove the supernatant with a pipette. ★Repeat the wash step with an additional 500 µl of Wash Buffer (make sure ethanol was added). Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer then remove the supernatant with a pipette.
Step 4 **Elution**	★Air-dry the DL Suspension Buffer pellet at room temperature or 37ºC for 10-15 minutes with the cap open. NOTE: Over drying the DL Suspension Buffer will decrease DNA fragment recovery. ★Add 10-20 µl of Elution Buffer[1], TE[2] or water[3] then vortex to resuspend the DL Suspension Buffer. Incubate the tube at 50ºC for 5 minutes. During incubation, vortex every 2 minutes. NOTE: For larger DNA fragments (>10 kb), increase incubation time to 10-15 minutes. ★Centrifuge for 1 minute at 10,000 x g to pellet the DL Suspension Buffer. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube. NOTE: Do not touch the DL Suspension Buffer pellet when transferring the supernatant. NOTE: Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%.

	[1]Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) [2]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. [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. DNA eluted in water should be stored at -20ºC to avoid degradation.

PCR Cleanup Protocol

Please read the entire instruction manual prior to starting.

Caution: During operation, always wear a lab coat, disposable gloves, and protective goggles.

Things to Do before Starting:

1. 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.
2. Set heating block or water bath at 50ºC.
3. It is not necessary to remove mineral oil or kerosene from the PCR sample prior to cleanup.

Additional Requirements: 1.5ml microcentrifuge tubes, absolute ethanol.

Step 1 PCR Cleanup Sample Preparation	★Transfer up to 100 µl of reaction product to a 1.5 ml microcentrifuge tube. Add 5 volumes of DL Buffer to 1 volume of the sample and mix by vortex. If the color of the mixture has turned from yellow to purple, add 10µl of Sodium Acetate (pH5.0) and mix thoroughly. This will adjust the pH and the color will return to yellow.
Step 2 DNA Binding	★Resuspend DL Suspension Buffer by vortex for 30 seconds. Add 10 µl of DL Suspension Buffer per 5 µg of DNA to the sample then mix the tube thoroughly by vortex. ★Incubate at room temperature for 10 minutes. During incubation, vortex the tube every 2-3 minutes. ★Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer then remove the supernatant using a pipette.

Step 3 **Wash**	★Add 500 µl of Wash Buffer (make sure ethanol was added) to the pelleted DL Suspension Buffer then resuspend the pellet by brief vortex. Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer. Remove the supernatant with a pipette. ★Repeat the wash step with an additional 500 µl of Wash Buffer (make sure ethanol was added). Centrifuge at 10,000 x g for 30 seconds to pellet the DL Suspension Buffer then remove the supernatant with a pipette.
Step 4 **Elution**	★Air-dry the DL Suspension Buffer pellet at room temperature or 37ºC for 10-15 minutes with the cap open. NOTE: Over drying the DL Suspension Buffer will decrease DNA fragment recovery. ★Add 10-20 µl of Elution Buffer[1], TE[2] or water[3] then vortex to resuspend the DL Suspension Buffer. Incubate the tube at 50ºC for 5 minutes. During incubation, vortex every 2 minutes. NOTE: For larger DNA fragments (>10 kb), increase incubation time to 10-15 minutes. ★Centrifuge for 1 minute at 10,000 x g to pellet the DL Suspension Buffer. Carefully transfer the supernatant containing the purified DNA to a new 1.5 ml microcentrifuge tube. NOTE: Do not touch the DL Suspension Buffer pellet when transferring the supernatant. NOTE: Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%. [1]Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) [2]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. [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. DNA eluted in water should be stored at -20ºC to avoid degradation.

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	**Agarose gel did not dissolve completely.** Ensure the agarose gel was melted/dissolved completely between 50-60ºC for 10-15 minutes, or until no gel is visible. If undissolved agarose remains in the sample, some DNA will be unrecoverable. DNA can be denatured if the incubation temperature exceeds 60ºC. If using more than 250 mg of agarose gel, separate it into multiple 1.5 ml microcentrifuge tubes. Mix the tube every 2 minutes during incubation to ensure the silica matrix remains in suspension
	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. 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. Repeating the elution step with an additional 10-20 µl of Elution Buffer will increase the yield by approximately 10-15%.
	pH Is Too High (the color of the mixture has turned from yellow to purple, pH>7.5). Add 10 µl of 3M Sodium Acetate (pH5.0) to the sample mixture then mix thoroughly. This will adjust pH and the color of the sample will return to yellow indicating the appropriate pH to facilitate DNA binding.
	Over-dried DL Suspension Buffer Pellet. Do not dry the DL Suspension Buffer pellet by vacuum centrifuge.
Eluted DNA Does Not Perform Well In Downstream Applications	**Incomplete Removal of DL Buffer.** Salt from DL Buffer may inhibit subsequent enzymatic reactions. Wash the DL Suspension Buffer pellet twice with Wash Buffer.
	Residual Ethanol Contamination. Following the wash step, dry the DL Suspension Buffer pellet at room temperature or 37ºC for 10-15 minutes with the cap open. Residual ethanol from the Wash Buffer will inhibit downstream applications and must be removed completely.