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PrimeWay Gel Extraction Kit/ PCR Purification Kit (KIT-9050)

PrimeWay Gel Extraction/PCR Purification Kit

Product No: KIT-9050

PrimeWay Gel Extraction/PCR Purification kit offers 2 applications in one kit. It is a simple procedure and uses a silica-based spin column to perform gel extraction or purify the DNA fragments from 65 bp to 15 kb within 20 minutes with a high recovery rate. This kit enables removal of primers, dNTPs, enzymes, salts, and short PCR products (< 65 bp). Thus, making it suitable for downstream processes such as DNA sequencing, PCR, in-vitro transcription, restriction mapping, cloning, and labelling applications.

For Research Use Only. Not for use in Diagnostic Procedures.

Kit Contents

No	Product	KIT-9050-10 10 preps	KIT-9050-50 50 preps	KIT-9050-250 250 preps
1	BD Buffer	7 mL	40 mL	200 mL
2	Wash Buffer	1.7 mL	8 mL	41 mL
3	Elution Buffer	1 mL	5 mL	24 mL
4	PrimeWay Gel/PCR Column	10 pcs	50 pcs	5 x 50 pcs
5	Collection Tube	10 pcs	50 pcs	5 x 50 pcs

Storage

This kit should be stored at room temperature (21 – 25 °C).

Product Specification

	PCR Product Purification	Gel Extraction
Binding capacity	20 µg	20 µg
Sample	≤ 100 µL PCR product	≤ 300 mg gel size
DNA fragment size	65 bp – 15 kb	65 bp – 15 kb
Recovery	Up to 100%	Up to 99%
Elution	40 µL	40 µL
Duration	~ 10 minutes	~ 20 minutes

Materials Supplied by Users

- ✓ Ethanol (96 – 100%)
- ✓ Centrifuge, at speed of 11,000 – 18,000 × g
- ✓ Vortex mixer
- ✓ Water Bath/ Dry Bath at 55 °C (Gel Extraction)
- ✓ 1.5 mL microcentrifuge tubes

Precautions for Users

- ✓ Some buffer of this kit contains irritants. Handle with care and avoid contact with skin. In case of contact, wash skin with plenty amount of water and seek medical attention.
- ✓ Always wear a lab coat, disposable gloves and surgical mask.

Before Start

- ✓ Set water bath/ dry bath to 55 °C.
- ✓ Add ethanol (96 – 100%) to Wash Buffer as following:

P/No	Ethanol to be added
KIT-9050-10	6.8 mL
KIT-9050-50	32 mL
KIT-9050-250	164 mL

Protocol – Gel Extraction

Sample	1. Excise **up to 300 mg gel slice** with a clean scalpel. ***Note**: Minimize the gel size by remove excess agarose gel.* 2. Transfer the gel slice into a new 1.5 mL microcentrifuge tube. 3. Add **500 µL BD Buffer** to the gel and vortex to mix. For > 2% agarose gels, add **1,000 µL BD Buffer**. 4. Incubate the mixture at 55 °C for 5 – 10 minutes or until the gel slice has been completely dissolved. Vortex the tube every 2 – 3 minutes. ***Note**: If the mixture turns violet, add 10 µL of 3M sodium acetate (pH 5.0), vortex to mix. The mixture will turn yellow.*
Binding	5. Cool the gel mixture to room temperature. 6. Place the **PrimeWay Gel/PCR Column** into the Collection Tube. Transfer **up to 800 µL the gel mixture** into the **PrimeWay Gel/PCR Column**. ***Note**: If gel mixture > 800 µL, repeat this step by loading the remaining volume after Step 7.* 7. Centrifuge at 11,000 × *g* for 30 seconds. Discard the flow-through and place the **PrimeWay Gel/PCR column** back to collection tube.
Washing	8. Add **750 µL Wash Buffer** into the **PrimeWay Gel/PCR Column.** ***Important!!** Make sure ethanol is added to the buffer prior to first use.* 9. Centrifuge at 11,000 × *g* for 30 seconds. Discard the flow-through and place the **PrimeWay Gel/PCR column** back to collection tube.
Drying	10. Dry the column by centrifuge at maximum speed (~ 18,000 x *g*) for 3 minutes. ***Note**: Make sure Wash Buffer is completely removed as it can inhibit downstream application.*

Elution	11. Place **PrimeWay Gel/PCR Column** into a new 1.5 mL microcentrifuge tube. 12. Add **40 µL Elution Buffer** to the center of the **PrimeWay Gel/PCR Column**. 13. Let the column stand for 1 minute. 14. To elute the DNA, centrifuge at maximum speed (~ 18,000 x *g*) for 1 minute.

Protocol – PCR Purification

Sample	1. Transfer **up to 100 µL PCR product** into a new 1.5 mL microcentrifuge tube. ***Note**: If >100 µL PCR product, separate into multiple tube to process* 2. Add **5 volumes BD Buffer** to 1 volume PCR product and mix by vortexing. *Example:* • *For 25 µL PCR product, add 125 µL of BD Buffer* • *For 50 µL PCR product, add 250 µL of BD Buffer*
Binding	3. Place the **PrimeWay Gel/PCR Column** into the Collection Tube. 4. Transfer the PCR mixture into the **PrimeWay Gel/PCR Column**. 5. Centrifuge at 11,000 × *g* for 30 seconds. Discard the flow-through and place the **PrimeWay Gel/PCR column** back to collection tube.
Washing	6. Add **750 µL Wash Buffer** into the **PrimeWay Gel/PCR Column**. ***Important!!** Make sure ethanol is added to the buffer during first use.* 7. Centrifuge at 11,000 × *g* for 30 seconds. Discard the flow-through and place the **PrimeWay Gel/PCR column** back to collection tube.
Drying	8. Dry the column by centrifuge at maximum speed (~ 18,000 x *g*) for 3 minutes. ***Note**: Make sure Wash Buffer is completely removed as it can inhibit downstream application.*
Elution	9. Place **PrimeWay Gel/PCR Column** into a new 1.5 mL microcentrifuge tube. 10. Add **40 µL Elution Buffer** to the center of the **PrimeWay Gel/PCR Column**. 11. Let the column stand for 1 minute. 12. To elute the purified DNA, centrifuge at max speed (~ 18,000 x *g*) for 1 minute.

Troubleshooting Guidelines

Problems	Possible Reason	Recommended Action
Low DNA yield	Reagents not prepared properly	Make sure ethanol (96 – 100%) have been added to Wash Buffer. Mix well before use.
	Incomplete dissolved of gel slice	• Increase incubation time. • Gel slice too big, separate the gel slice into multiple tube
	Inefficient DNA elution	Ensure Elution Buffer is added to the center of column membrane and completely absorbed before centrifugation.
	DNA sample with > 5 kb	Use pre-heated Elution Buffer (60 °C) for elution step.
Non-specific bands	Denaturation of sample	Incubate the eluent at 95°C for 2 minutes. Let the solution cool down gradually at room temperature to promote reannealing of the denatured DNA
Problem in the downstream processes	Presence of salt residue	Perform additional Washing step using Wash Buffer.
	Ethanol carry-over	Increase centrifugation time with additional 3 minutes to ensure the Wash Buffer is completely removed.

Please contact us at https://base-asia.com/contact/ for more information.