imagen

ImaSpin® Genomic DNA Extraction Kit –Gel & PCR

For purification of concentrate DNA fragments from agarose gel, PCR or other enzymatic reactions

Precautions

I. Handling Requirements
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

II. Equipment and Reagents to Be Supplied by User

- Ethanol (96–100%)*
- 1.5 ml microcentrifuge tubes
- Pipet tips with aerosol barrier
- Vortexer
- Microcentrifuge (with rotor for 1.5 ml tubes) may be required for some samples

* Do not use denatured alcohol, which contains other substances such as methanol or methylethylketone.

III. Waste Handling

- Treat waste with the country, federal, state and local regulations.

IV. Important points before use

- Do not use the product if it has expired.
- Add absolute ethanol (see the bottle label for volume) to IW2 Buffer then mix by shaking for a few seconds and tick the checkbox of the label on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Kit Contents

IDF Buffer
IRW2 Buffer (Add Ethanol)
Elution Buffer
IG Column
2 ml Collection Tubes

Storage and Stability:

This kit should be stored at room temperature.

Description

ImaSpin® Genomic DNA Extraction Kit –Gel & PCR is designed by patented technology to recover or concentrate DNA fragments from agarose gel, PCR, or other enzymatic reactions in one convenient product. Salts, enzymes and unincorporated nucleotides can be effectively removed from the reaction mixture, without phenol extraction or alcohol precipitation. Typically, recoveries are up to 90% for Gel Extraction and up to 95% for PCR Clean Up. The eluted DNA is ready for use in PCR, Fluorescent or Radioactive Sequencing, Restriction Enzyme Digestion, DNA Labeling and Ligation.

ImaSpin® Genomic DNA Extraction Kit –Gel & PCR Test Data

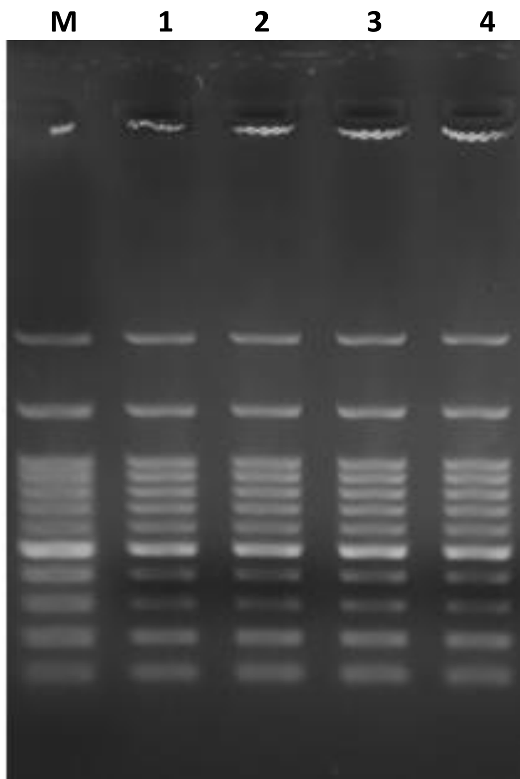

Fig 1. Competition of the Recovery of ImaSpin® Genomic DNA Extraction Kit –Gel & PCR & Competitive brand Q

Gel slice DNA fragments ranging from 100 bp- 3 kb were extracted using the ImaSpin® Genomic DNA Extraction Kit –Gel & PCR & & Competitive brand Q. The purified DNA from a 50 µl eluate was analyzed by electrophoresis on a 1% agarose gel.
M = 1 Kb DNA Ladder
1-2 = Competitive brand Q
3-4 = ImaSpin® Genomic DNA Extraction Kit –Gel & PCR

Preparation before using

Add absolute ethanol (see the bottle label for volume) to IRW2 Buffer prior to initial use.

PCR Clean Up Protocol Procedure

1. Transfer up to 100 µl of reaction product to a 1.5 microcentrifuge tube.
2. Add 5 volumes of IDF Buffer to 1 volume of the sample and mix by vortex.
3. Place an IG Column in a 2 ml Collection Tube.
4. Transfer the sample mixture to the IG Column.
5. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds.
6. Discard the flow-through then place the IG Column back in the 2 ml Collection Tube.
7. Add 600 µl of IRW2 Buffer (make sure ethanol was added) into the CENTER of the IG Column.
8. Let stand for 1 minute at room temperature.
9. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds.
10. Discard the flow-through and place the IG Column back in the 2 ml Collection Tube.
11. Centrifuge for 3 minutes at 13,000 rpm (10,000 x g) to dry the column matrix.
12. Transfer the dried IG Column to a new 1.5 ml microcentrifuge tube. •
13. Add 20-50 µl of Elution Buffer or TE into the CENTER of the column matrix.
    NOTE: If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
14. Let stand for at least 2 minutes to ensure the Elution Buffer is completely absorbed.
15. Centrifuge for 2 minutes at 13,000 rpm (10,000 x g) to elute the purified DNA.
    NOTE: Using pre-heated Elution Buffer (60 °C) is recommended for eluting DNA fragments >5kb

Gel Extraction Protocol Procedure

1. Excise the agarose gel slice containing relevant DNA fragments and remove any extra agarose.
   NOTE: Using TAE buffer for gel formation is recommended for optimal DNA recovery.
2. Transfer up to 300 mg of the gel slice to a 1.5 ml microcentrifuge tube then add 500 µl of IDF Buffer and vortex.
3. Incubate at 55-60 °C for 10-15 minutes to ensure the gel slice has been completely dissolved.
   NOTE: During incubation, invert the tube every 2-3 minutes.
4. Place the IG Column in a 2 ml Collection Tube then transfer 800 µl of the sample mixture to the IG Column.
5. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds then discard the flow-through.
6. Place the IG Column back in the 2 ml Collection Tube. NOTE: If the sample mixture is more than 800 µl, repeat the DNA Binding Step.
7. Add 600 µl of IRW2 Buffer (make sure ethanol was added) into the IG Column and let stand for 1 minute.
8. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds then discard the flow-through.
9. Place the IG Column back in the 2 ml Collection Tube.
10. Add 600 µl of IRW2 Buffer (make sure ethanol was added) into the IG Column and let stand for 1 minute.
11. Centrifuge at 13,000 rpm (10,000 x g) for 30 seconds then discard the flow-through.
12. Place the IG Column back in the 2 ml Collection Tube.
13. Centrifuge at 13,000 rpm (10,000 x g) for 3 minutes to dry the column matrix.
14. Transfer the dried IG Column to a new 1.5 ml microcentrifuge tube.
15. Add 20-50 µl of Elution Buffer or TE into the CENTER of the column matrix.
    NOTE: If higher DNA yield required, repeat the DNA Elution Step to increase DNA recovery.
16. Let stand for at least 2 minutes to ensure the Elution Buffer is completely absorbed.
17. Centrifuge for 2 minutes at 13,000 rpm (10,000 x g) to elute the purified DNA.
    NOTE: Using pre-heated Elution Buffer (60 °C) is recommended for eluting DNA fragments >5kb.

imagen

Troubleshooting

Problem	Possible Reasons/Solution
Low Yield	➢ Ensure absolute ethanol was added to IRW2 Buffer and close the bottle tightly after each use to avoid ethanol evaporation. ➢ Ensure Elution Buffer or water is added into the CENTER of the column matrix. ➢ Elute twice to increase yield. **Gel slice did not dissolve completely** ➢ The Gel slice was too big. If using more than 300 mg of gel slice, separate it into multiple tubes. ➢ Use ≤2 % agarose gel to ensure optimal dissolution efficiency and DNA yield. ➢ Raise the incubation temperature to 60 °C and extend the incubation time. **Incorrect DNA Elution Step** ➢ Ensure that the Elution Buffer is completely absorbed after being added to the center of the IG Column. **Incomplete DNA Elution** ➢ If DNA fragments are larger than 5 kb, use pre-heated Elution Buffer (60-70 °C) to improve the elution efficiency.
DNA doesn't perform well in downstream applications	**DNA was denatured (a smaller band appeared on gel analysis)** ➢ Incubate the eluted DNA at 95 °C for 2 minutes, and then cool down slowly to re-anneal the denatured DNA.
Eluted DNA does not perform well in downstream applications	➢ Following the Wash Step, dry the IG column by incubate at 60 °C for 5 minutes. ➢ Use fresh sample, long term storage ample may result in fragmentation of genomic DNA. ➢ 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 using water for elution, ensure the water pH is between 7.5 and 8.5. ddH2O should be fresh as ambient CO_2 can quickly cause acidification. DNA eluted in water should be stored at -20 °C to avoid degradation **Low A260/A230** ➢ In the wash step, repeat the 600 µl of IRW2 Buffer addition and let stand for 1 minute.