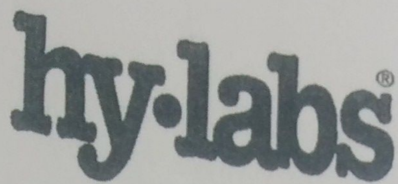

Item No: EX-GP200 (200rxns)

Gel/PCR Extraction Kit

For research use only

Content

Content	EX-GP200
Binding Buffer BD	80 ml
Wash Buffer PE	20 mlx3
Elution Buffer (10 mM Tris-HCl, pH 8.5)	10 ml
Spin Columns	200 each

Description

Gel /PCR Extraction Kit is designed to extract and purify DNA from standard or low-melt agarose gels in either Tris acetate (TAE) or Tris borate (TBF) and from PCR and other enzymatic reaction mixtures.This membrane-based system, which can bind up to 40μg DNA, allows recovery of isolated DNA in as little as 20 minutes, depending on the number of samples processed and the protocol used. The purified DNA can be used for automated fluorescent DNA sequencing, cloning, labeling, restriction enzyme digestion or in vitro transcription/translation without further manipulation.

Applications

Fast and efficient extraction of high purity DNA ideal for use in all conventional molecular biology procedures including:

- conventional restriction digestion
- automated fluorescent or radioactive sequencing
- PCR
- DNA Sequencing
- in vitro transcription

Feature

- **Fast** - procedure takes only 15 min.
- **High Efficient** – up to 85% recoveries in the range of 50bp-40kb.
- **High purity** - $OD_{260/280}$=1.7-1.9. Purified DNA is ready for downstream application such as PCR, restriction digestion.

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❖ PCR and DNA Fragment Extraction

1. Transfer the PCR reaction mix or other enzymatic reaction mix to a new 1.5ml microcentrifuge tube. And add a 1:1 volume of Binding Buffer BD to the mixture (e.g. for every 100 μl of reaction mixture, add 100 μl of Binding Buffer). Mix thoroughly.
2. Transfer up to 800 μl of the solution from step 1 to the spin column. Incubate for 2 min.
3. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through.
   Note. If the total volume exceeds 800 μl, the solution can be added to the column in stages. After the addition of 800 μl of solution, centrifuge the column for 30-60 s and discard flow-though. Repeat until the entire solution has been added to the column membrane.
4. Add 500 μl of Wash Buffer PE to the column. Centrifuge for 1 min at 12,000 rpm. Discard the flow-through and place the purification column back into the collection tube.
   Note Wash Buffer PE must previously diluted with ethanol(96-100%).
5. Repeat step 4 again.
6. Centrifuge the empty column for an additional 3 min to completely remove any residual wash buffer.
   Note. This step is essential as the presence of residual ethanol in the DNA sample may inhibit subsequent reactions.
7. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
8. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 μl Elution Buffer TE (prewarm to 60℃)directly to the center of the column without touching the membrane. Incubate at room temperature for 2 min.
   Note • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 μl does not significantly reduce the DNA yield. However, elution volumes less than 10 μl are not recommended.
   • If DNA fragment is >10 kb, prewarm Elution Buffer to 60°C before applying to Column.
   • If the elution volume is 10 μl and DNA amount is >5 μg, incubate column for 1 min at room temperature before centrifugation.
9. Centrifuge for 1 minute at 12,000rpm. Discard the columns and store the microcentrifuge tube containing the eluted DNA at −20°C.
   Note • Elution buffer can be replaced by deionized water. But the PH should be 8.0-8.5.
   • Prewarm Elution Buffer TE to 60℃ can increase the yield of genomic DNA.

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Gel Extraction Kit can be stored for up to 12 months at room temperature (15-25°C) or at 4°C for storage periods longer than 12 months. Any precipitate in the buffers can be re-dissolved by incubating at 37°C before use.

Quality Control

The kit is tested in the purification of 50 bp and 1 kb PCR products according to the protocol. The quality of the purified DNA is evaluated spectrophotometrically, by agarose gel electrophoresis, digestion with restriction enzymes and automated fluorescent sequencing.

Note

- Prior to the initial use of the kit, dilute the Wash Buffer PE with ethanol (96-100%):

	EX-GP200
Wash Buffer PE	20ml ×3
Ethanol	80 ml ×2
Total Volume	100 ml ×2

After the ethanol has been added, mark the check box on the bottle to indicate the completed step.

- Examine the Binding Buffer BD for precipitates before each use. Re-dissolve any precipitate by warming the solution to 37°C and cooling to 25°C.
- Wear gloves when handling the Binding Buffer BD as this solution contains irritants.
- If extracted DNA will be used directly for sequencing, freshly prepared electrophoresis buffers should be used both for gel preparation and for gel running
- All centrifugations should be carried out in a table-top microcentrifuge at>12000 g (10,000-14,000 rpm, depending on the rotor type)

Protocol

❖ **Gel extraction**

1. Weigh a 1.5ml microcentrifuge tube for each DNA fragment to be isolated and record the weight.
2. Excise gel slice containing the DNA fragment using a clean scalpel or razor blade. Cut as close to the DNA as possible to minimize the gel volume. Place the gel slice into a pre-weighed 1.5 ml tube and weigh. Record the weight of the gel slice.
   Note If the purified fragment will be used for cloning reactions, avoid damaging the DNA through UV light exposure. Minimize UV exposure to a few seconds or keep the gel slice on a glass or plastic plate during UV illumination.

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3. Add Binding Buffer BD at a ratio of 10μl of solution per 10mg of agarose gel slices.
4. Incubate the gel mixture at 50-60°C for 7-10 min or until the gel slice is completely dissolved. Mix the tube by inversion every few minutes to facilitate the melting process. Ensure that the gel is completely dissolved.

   Note • Check the color of the solution. A yellow color indicates an optimal pH for DNA binding. If the color of the solution is red, add 10 μl of 3 M sodium acetate, pH 5.2 solution and mix. The color of the mix will become yellow.

   • High concentration gels (>2% agarose) or large gel slices may take longer than 10 minutes to dissolve.
5. Transfer the dissolved gel mixture to the Spin Columns assembly and incubate for 2 minute at room temperature.
6 Centrifuge the Spin Columns assembly in a microcentrifuge at ~12,000 rpm for 1 minute, then discard the flow-through.
7. Wash the columns by adding 500 μl of Wash Buffer PE to the Columns. Centrifuge the columns assembly for 1 minute at ~12,000 rpm , then discard the flow-through.

   Note Wash Buffer PE must previously diluted with ethanol(96-100%).
8. Repeat step 7 again.
9. Centrifuge the Columns for an additional 3 min to completely remove residual wash buffer.

   Note. This step is essential to avoid residual ethanol in the purified DNA solution. The presence of ethanol in the DNA sample may inhibit downstream enzymatic reactions.
10. Empty the Collection Tube and recentrifuge the column assembly for 1 minute with the microcentrifuge lid open (or off) to allow evaporation of any residual ethanol.
11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 μl Elution Buffer TE (prewarm to 60℃)directly to the center of the column without touching the membrane. Incubate at room temperature for 2 min.

    Note • For low DNA amounts the elution volumes can be reduced to increase DNA concentration. An elution volume between 20-50 μl does not significantly reduce the DNA yield. However, elution volumes less than 10 μl are not recommended.

    • If DNA fragment is >10 kb, prewarm Elution Buffer to 60°C before applying to column.

    • If the elution volume is 10 μl and DNA amount is >5 μg, incubate column for 1 min at room temperature before centrifugation.
12. Centrifuge for 1 minute at ~12,000 rpm. Discard the columns and store the microcentrifuge tube containing the eluted DNA at −20°C.

    Note • Elution buffer can be replaced by deionized water. But the PH should be 8.0-8.5.

    • Prewarm Elution Buffer TE to 60℃ can increase the yield of genomic DNA.

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