HiPer® Gel Extraction Teaching Kit (Solution Based)

Product Code: HTBM011

Number of experiments that can be performed: 10

Duration of Experiment:

Agarose Gel Electrophoresis: 1 hour
Protocol: 1 hour
Agarose Gel Electrophoresis: 1 hour

Storage Instructions:

- The kit is stable for 12 months from the date of manufacture
- Control DNA, DNA Sample and Wash Solution should be stored at -20°C
- Store Glass Powder Suspension, Chaotropic Salt Solution and 6X Gel Loading Buffer at 2-8°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To learn the technique of DNA purification from agarose gel using silica based technique.

Introduction:

Gel extraction is a technique used to isolate a DNA fragment from an agarose gel following agarose gel electrophoresis. The Gel Extraction Teaching Kit (Solution Based) for DNA isolation provides a convenient method for extracting DNA from agarose gels, for recovering DNA from Polymerase Chain Reactions and for concentration of DNA without ethanol precipitation. Upto 75-80% of the DNA can be recovered after extraction.

Principle:

In HiPer® Gel Extraction Teaching Kit (Solution Based) DNA is purified from agarose gel by using silica. This method involves the use of chaotropic salts that can solubilize agarose at 55ºC. In the presence of high concentrations of chaotropic salts, DNA binds to glass powder particles and elutes when the salt concentration is reduced. As the salt concentration reduces, rehydration of DNA breaks the bonding between the glass powder matrix and the DNA. The glass powder with adsorbed DNA when washed with the wash buffer containing ethanol helps in the removal of salts and impurities from the original sample. The pure DNA can be eluted in Elution Buffer at 55ºC. This isolation method of DNA is faster and easier to perform than the other organic solvent based extraction methods.

Kit Contents:

This Kit demonstrates extraction of DNA from standard agarose using silica based technique.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials Provided	Quantity 10 expts	Storage
1	TKC016	Control DNA	0.11 ml	-20ºC
2	TKC127	DNA Sample	0.11 ml	-20ºC
3	DS0006	Glass Powder Suspension (GPS)	0.15 ml	2-8ºC
4	DS0007	Chaotropic Salt Solution (CSS)	20 ml	2-8ºC
5	DS0008	Wash Solution	20 ml	-20ºC
6	DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	0.6 ml	R T
7	MB002	Agarose	9.6 g	R T
8	ML016	50X TAE	240 ml	R T
9	ML015	6X Gel Loading Buffer	0.06 ml	2-8ºC
10	PW1139	Collection Tubes, Polypropylene (2.0 ml)	30 Nos.	R T

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Reagents: Sterile distilled water, Ethidium bromide (10 mg/ml)
Other requirements: UV Spectrophotometer, Tabletop microcentrifuge (with rotor for 2.0 ml tubes), Electrophoresis apparatus, UV Transilluminator, Heating block or Water bath, Vortex mixer, Clean razor blade, Spatula, Micropipettes, Tips

Storage:

HiPer® Gel Extraction Teaching Kit (Solution Based) is stable for 12 months from the date of manufacture without showing any reduction in performance. On receipt, store Glass Powder Suspension, Chaotropic Salt Solution and 6X Gel Loading Buffer at 2-8ºC. Control DNA, DNA Sample and Wash Solution should be stored at –20ºC. Other reagents can be stored at room temperature (15-25ºC).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment.
2. Preheat the heating block or water bath at 55ºC.
3. **Glass Powder Suspension:** Seal it with parafilm while storing at 2-8ºC. Mix well before using. If the Glass Powder Suspension loses liquid and dries out, add sterile distilled water* such that the Glass Powder Suspension accounts for approximately two-third of the total volume.
4. In gel extraction procedure since agarose gel electrophoresis is to be carried out twice, 1X TAE buffer can be reused.

   * Molecular biology grade water is recommended (Product code: ML024)

Procedure:

Read the important instructions before starting the experiment.

A. Electrophoresis of DNA to be purified:

1. Prepare 0.8% of agarose (as given below in Agarose Gel Electrophoresis). Allow the solution to cool down to about 55-60ºC. Add 0.5 µl of Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

2. To prepare samples for gel electrophoresis, add 2 µl of 6X gel loading buffer for every 10 µl of DNA sample. Mix well and load the sample.

3. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

   Note: Store the 1X TAE tank buffer for later use.

4. Excise DNA bands from the Ethidium bromide stained gel using a clean razor blade or scalpel blade using 312 nm UV light and store it in a pre-weighed plastic micro centrifuge tube at 4ºC.

   NOTE: Cut the DNA fragment, minimizing the amount of agarose in the slice. Also minimize the amount of time the DNA is exposed to UV light by having sterile pre-weighed labeled tubes ready for the slices to avoid degradation of DNA.

5. Quantitate the concentration of DNA using a spectrophotometer or estimate the concentration by comparing its intensity with that of a DNA ladder of known concentration.

B. Gel Extraction:

1. Weigh the micro centrifuge tube again after adding the gel slice into it. Determine the approximate weight of the gel slice and accordingly add 3 volumes of Chaotropic Salt Solution per gel slice volume.

Incubate at 55°C for 10 minutes with intermittent mixing for every 2-3 minutes so that the agarose dissolves completely.
For e.g. 100 mg of agarose gel slice requires 300 µl of Chaotropic Salt Solution.

2. Vortex the glass powder suspension thoroughly. Add 10 µl of **well-mixed** glass powder suspension. Mix well and incubate at room temperature for 10 minutes with intermittent mixing every 2-3 minutes so that the glass powder gets resuspended completely in the solution. Centrifuge at 10,000 rpm for 1 minute. Discard the supernatant.

3. Add 500 µl of Wash Solution to the glass powder pellet and resuspend the glass powder pellet completely by gentle pipetting or pulse vortexing. Centrifuge at 10,000 rpm for 1 minute and discard the supernatant. Repeat the washing procedure for two more times. During each wash, the pellet should be resuspended completely.

4. After last wash, remove the supernatant completely using a micropipette. Spin the tube at 10,000 rpm for additional 1 minute to remove the traces of Wash Solution. Air-dry the pellet for 5 minutes.

   NOTE: Avoid over drying of the pellet as this may lead to poor recovery of DNA.

5. Resuspend the glass powder pellet in 50 µl of Elution Buffer. Incubate at 55°C for 10 minutes with intermittent mixing.

6. Centrifuge at 10,000 rpm for 1 minute and then carefully transfer the supernatant containing the eluted DNA to a new collection tube.

7. Centrifuge once again at 10,000 rpm for 1 minute to remove the traces of glass powder suspension completely [this also allows easy evacuation of the supernatant].

8. Carefully transfer the supernatant containing the eluted DNA to a new tube.

Storage of the eluate with purified DNA: The eluate contains pure DNA. For short term storage (24-48 hours) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample, which may cause denaturation of DNA.

Agarose Gel Electrophoresis:

Preparation of 1X TAE: To prepare 500 ml of 1X TAE buffer, add 10 ml of 50X TAE Buffer to 490 ml of sterile distilled water*. Mix well before use.

Preparation of agarose gel: To prepare 50 ml of 0.8% agarose gel, add 0.4 g agarose in 50 ml 1X TAE buffer in a glass beaker or flask. Heat the mixture on a microwave or hot plate by swirling the glass beaker/ flask occasionally, until agarose dissolves completely (Ensure that the lid of the flask is loose to avoid buildup of pressure). Allow the solution to cool to about 55-60°C. Add 0.5µl Ethidium bromide, mix well and pour the gel solution into the gel tray. Allow the gel to solidify for about 30 minutes at room temperature.

Loading of the DNA samples: To prepare sample for electrophoresis, add 2 µl of 6X gel loading buffer to 10 µl of DNA sample. Mix well by pipetting and load the sample into the well. Load the Control DNA after extracting the DNA sample.

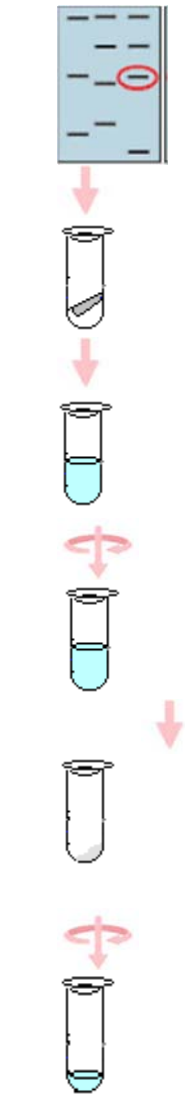
Electrophoresis: Connect the power cord to the electrophoretic power supply according to the conventions: Red-Anode and Black- Cathode. Electrophorese at 100-120 volts and 90 mA until dye markers have migrated an appropriate distance, depending on the size of DNA to be visualized.

* Molecular biology grade water is recommended (Product code: ML024).

Quantitation of DNA:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute the samples and to calibrate the spectrophotometer. Measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm.
Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor.

Flowchart:



Agarose gel electrophoresis

- Electrophorese the DNA samples on low melting agarose

Sample Preparation

- Excise the DNA band of interest from the gel
- Weigh the gel slice containing the DNA band

Gel Extraction

- Add 3 volumes of Chaotropic Salt Solution per gel slice volume
- Incubate at 55^{0}C for 10 minutes with intermittent mixing
- Add 10 µl of Glass Powder Suspension
- Incubate at room temperature for 10 minutes with intermittent mixing
- Centrifuge at 10,000 rpm for 1 minute

Wash to remove residual contaminants

- Add 500 µl of Wash Solution to the glass powder pellet
- Resuspend by gentle pipetting and centrifuge at 10,000 rpm for 1 minute, discard the supernatant
- Repeat the washing procedure two more times
- After final wash, centrifuge for additional 1 minute at 10,000 rpm
- Air-dry the pellet for 5 minutes

DNA Elution

- Resuspend the glass powder pellet in 50 µl of Elution buffer
- Incubate at 55^{0}C for 10 minutes with intermittent mixing
- Centrifuge at 10,000 rpm for 1 minute and transfer the supernatant containing the eluted DNA to a new collection tube
- Repeat the above step to elute pure DNA

Pure DNA

Observation and Result:

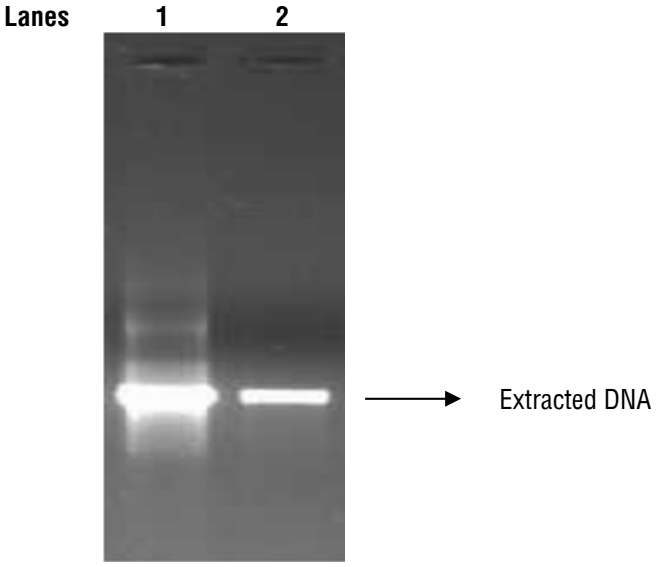


Fig 1: Gel image of extracted DNA from agarose gel

Lane 1: Control DNA
Lane 2: Extracted DNA

Table 2: Absorbance of the extracted genomic DNA at 260 nm and 280 nm

Sample	Dilution Factor	A_{260}	A_{280}	A_{260}/A_{280}	Concentration (µg/ml)
1					
2					
3					

Calculate the concentration of isolated DNA using following formula:

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Interpretation:

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct background absorbance. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. The concentration of DNA is calculated by the following formula:

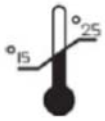
Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor

Troubleshooting Guide:

Sr. No.	Problem	Probable Cause	Solution
1	Very less amount of DNA or no DNA obtained	More volumes of Glass Powder Suspension used than required	Using more volumes of Glass Powder Suspension could be detrimental to DNA yield and may result in elution of very less amount of DNA or no DNA. Hence it is recommended to use volumes that correspond to the amount of DNA
		Glass powder settles at the bottom of the tube	The glass powder should not be allowed to settle at the bottom. Glass powder: DNA mixture should be mixed well every 2-3 minutes for proper binding
		Traces of residual Chaotropic Salt Solution	Prior to the elution of DNA remove all the residual Chaotropic Salt Solution by washing with ethanol, this is important because if it is present in the final elution, it may lower the recovery of DNA or inhibit enzymes used in subsequent reactions
		Pellet is too dry	Do not dry the pellet for too long. Resuspend the pellet in Elution Buffer and incubate at 55°C for 5 minutes
2	Agarose gel was not completely solubilized	Incorrect incubation temperature and time	The incubation temperature for solubilization of the gel should be 55°C. The incubation time can be increased till the gel completely dissolves
		Insufficient amount of Chaotropic Salt Solution added	The amount of Chaotropic Salt Solution to be added should be 3 volumes
3	Incomplete dissolution of DNA	Over drying of the pellet	Pellet should not be dried for more than 5 minutes
		Traces of Wash Buffer left in the tube	Wash Buffer has to be completely removed or else traces of Wash Buffer remaining in the tube may interfere with the enzymatic reactions

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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