Gel Bind Buffer (HG)

Product Name	Product Code	Kit Packing
Gel Bind Buffer (HG)	DS0023-250ML	250 ml

Intended Use

Recommended for binding of DNA while purification from agarose gels.

Introduction: Gel Bind Buffer (HG) is a buffer solution used for binding of DNA to the column membrane.

Application: DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer

Properties:

Appearance: Yellow colored solution
Clarity: Clear and free of particles
DNase & RNase: None detected

Suitability Test: This reagent has been tested and is suitable for binding of DNA to the column membrane.

Storage conditions: Gel Wash Concentrate (BIW) has to be stored at 15 - 25°C. The shelf- life of this solution in 12 months.

General Preparation Instructions

1. Ensure that clean & dry tubes and tips are used for the procedure.
2. Preheat heating block or water bath to 55-60°C.
3. **Thoroughly mix reagents**
   Examine the solutions for any kind of precipitation. If any solution forms a precipitate, warm at 55-65°C until the precipitate dissolves completely and allow it to cool to room temperature (15-25°C) before use.
4. Only up to 400 mg of agarose gel slice can be processed per column.
5. **Gel Wash Buffer (BIW) should be freshly prepared before the experiment:**
   Dilute Gel Wash Buffer Concentrate (BIW) (DS0030) in the ratio 1:4 using ethanol (96-100 %) and mix thoroughly.
   For example, to 1 ml of Gel Wash Buffer Concentrate (BIW), add 4 ml of ethanol (96-100%).

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Specimen Collection and Handling

Collect DNA from agarose gel. Store DNA gel at 2-8°C. Bring DNA to room temperature before use.

Types of Specimen

Samples: DNA in agarose gel

Procedure

NOTE: The yellow color of Gel Bind Buffer (HG) (DS0023) signifies a pH of ≤7.5.

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments.

   NOTE: Any type or grade of agarose can be used, but it is strongly recommended that the running buffer (either TAE buffer or TBE buffer) should be fresh. The pH of the buffer may increase by reusing the buffer, which may reduce the final yield.

2. After adequate separation of bands has occurred, excise the DNA bands from the ethidium bromide stained gel with a clean razor blade or scalpel blade using 312 nm UV light and place it in a clean 2.0 ml capped centrifuge tube.

   NOTE: The size of the gel slice should be minimized by removing extra agarose.

3. Determine the weight of the gel slice and accordingly add three volumes of Gel Bind Buffer (HG) (DS0023) per gel slice volume. Incubate the mixture at 55-60°C for 7 minutes or until the gel has completely melted. Mix the contents of the tube after every 2-3 minutes so that the agarose is completely dissolved.

   NOTE: For example, 100 mg of agarose gel slice requires 300 µl of Gel Bind Buffer (HG). Make sure that the agarose gel slice is solubilized completely.

4. **Load lysate onto HiElute Miniprep Spin Column**
   Apply the Gel/Gel Binding Buffer mixture (obtained from the above step) to a HiElute Miniprep Spin Column (Capped) and centrifuge at 10,000 x g (≈12,000 rpm) for 1 minute at room temperature (15-25°C)**.**

   NOTE: Approximately 700 µl of sample mixture can be loaded at a time in the HiElute Miniprep Spin Column (Capped). In case of sample volume larger than 700 µl, discard the flow-through obtained, add rest of the lysate to the column and repeat the spin.

5. Discard the flow-through and place the column back into the same collection tube.

6. Add 300 µl of Gel Bind Buffer (HG) into the column and centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature (15-25°C) to wash the membrane. Discard the flow-through and reuse the collection tube.

7. **Wash**
   (Freshly prepare the Gel Wash Buffer (BIW) as indicated in General Preparation Instructions)
   Place the column into the same collection tube and add 700 µl of diluted Gel Wash Buffer (BIW) (DS0030). Centrifuge for 1 minute at 10,000 x g (≈12,000 rpm) at room temperature (15-25°C). Discard the flow-through and reuse the collection tube.

   OPTIONAL: Repeat Step 7 with another 700 µl of diluted Wash Solution (BIW). Discard the flow-through liquid and reuse the collection tube.

NOTE: The second wash step is for any salt-sensitive downstream applications.

8. Centrifuge the empty column for 2 minutes at maximum speed ≈13,000 rpm to dry the column membrane.

   NOTE: This drying step is critical for removal of residual ethanol completely.

9. **DNA Elution**
   Place the column into a new 2.0 ml uncapped collection tube and add 30-50 µl of Elution Buffer (ET) (10mM Tris-Cl, pH 8.5) (DS0040) (depending on the desired concentration of the final product) directly onto the column membrane. Incubate at room temperature (15-25°C) for 1 minute. Centrifuge for 1 minute at maximum speed ≈13,000 rpm to elute the DNA.

   NOTE: The eluate represents approximately 70% of the bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

10. Transfer the eluate to a fresh capped 2 .0 ml collection tube for longer DNA storage.

Storage of the eluate with purified DNA: The eluate contains pure DNA. For short-term storage (24-48 hrs) 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 denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Warning and Precautions

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good laboratory practices while handling samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

1. The yield of DNA depends upon the volume of starting material used.

Performance and Evaluation

Each lot of Gel Bind Buffer (HG) is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Recovery
Gel with DNA	80-90 %

Safety Information

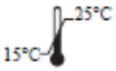
The Gel Bind Buffer (HG) contains irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Technical Assistance

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



Storage temperature

Do not use if package is damaged

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