

# **peqGOLD Gel Extraction Kit (Classic Line)**

**– Instruction Manual –**

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## Introduction

The peqGOLD family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is the new HiBind<sup>®</sup> matrix that specifically, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

Gel purification of DNA is a common technique for isolation of specific fragments from reaction mixtures. However, most methods either fail to completely remove agarose (which can lead to problems in downstream manipulations), shear the DNA, or result in very low yields. The peqGOLD Gel Extraction Kit uses HiBind<sup>®</sup> technology to recover DNA bands between 50 bp and 40 kb from all grades of agarose gel in yields exceeding 85%. The DNA band of interest is excised from the gel, dissolved in Binding Buffer, and applied to a HiBind<sup>®</sup> DNA spin-column. Following a rapid wash step, DNA is eluted with deionized water (or low salt buffer) and ready for further applications. The product is suitable for ligations, PCR sequencing, restriction digestion, or various labeling reactions.

## Benefits

- ! Speed - DNA recovery from agarose in less than 15 min.
- ! Reliability - optimized buffers guarantee pure DNA.
- ! Safety - No organic extractions required.
- ! Quality - purified DNA suitable for any application.

## Binding Capacity

Each HiBind<sup>®</sup> DNA column can bind up to 30 µg DNA.

## Kit Components

peqGOLD Gel Extraction Kit	5 Purifications	50 Purifications	200 Purifications
Product Number (C Line)	12-2501-00	12-2501-01	12-2501-02
<b>Components</b>			
HiBind <sup>®</sup> DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
XP2 Binding Buffer	3 ml	30 ml	120 ml
SPW Buffer	5 ml	25 ml	3 x 25 ml
Elution Buffer	1 ml	10 ml	30 ml
Instruction manual	1	1	1

## Storage and Stability

All peqGOLD Gel Extraction Kit components are stable for at least 12 months from the date of purchase when stored at room temperature (22 – 25 °C). Ensure that the bottle of Binding Buffer is tightly capped when not in use.

## Before Starting

Briefly examine this booklet and become familiar with each step. Prepare all components and have the necessary materials ready before starting.

! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-2501-00	Add 20 ml 100% EtOH to 5 ml Wash Buffer
Kit 12-2501-01	Add 100 ml 100% EtOH to 25 ml Wash Buffer
Kit 12-2501-02	Add 3 x 100ml 100% EtOH to 3 x 25ml Wash Buffer

Store diluted DNA Wash Buffer at room temperature.

! All steps must be carried out at room temperature.

## peqGOLD Gel Extraction Protocol

### *Extraction of DNAs from Agarose gels*

Materials required, but not supplied:

- ! 100 % Ethanol
- ! Sterile deionized water (optional)
- ! Sterile 1.5 ml centrifuge tubes

### 1. DNA Separation

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

*Any type or grade of agarose may be used. It is strongly recommended to use fresh TAE buffer as running buffer. Do not reuse running buffer as its pH is increased and will reduce yields. Fresh TBE may also be used, but usually gives lower yields.*

### 2. Excision of DNA Band

When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a UV light box ensuring not to take more agarose gel as necessary. Avoid more than 30 seconds exposure of UV light to the DNA. Transfer the gel slice to a fresh 1.5 ml microcentrifuge tube.

*Always use protective eye-ware or better a UV shield when working with UV light.*

### 3. Delution of Agarose

Determine the approximate volume of the gel slice by weighting it in the 1.5 ml microfuge tube. Assuming a density of 1 g/ml of gel, the volume of gel is derived as follows: a gel slice of mass 0.2 g will have a volume of 0.2 ml. Add equal volume of Binding Buffer. Incubate the mixture at 55 °C – 65 °C for 7 min or until the gel has completely melted.

**Important:** *Monitor the pH of the Gel/Binding buffer mixture after the gel completely dissolved. DNA yield will significantly decreased when pH > 8.0. If the color of the mixture become orange or red, Add 5 µl of 5M sodium acetate, pH 5.2 to bring the pH down. After this adjustment, the color of the gel/Binding buffer mixture should be light yellow.*

#### 4. Load and bind

Place a fresh HiBind® spin column in a 2 ml collection tube and add 700 µl of the DNA/agarose solution. Centrifuge the spin column / collection tube assembly for 1 min at 10'000 x g. Discard the flow-through and keep the collection tube for further steps.

For volumes higher than 700 µl load the column again and centrifuge successively, 700 µl at a time. Each HiBind® extraction column has a total capacity of 25-30 µg DNA. If the expected yield is higher, divide the sample into the appropriate number of columns.

#### 5. Wash I

Discard liquid and add 300µl Binding Buffer. Centrifuge at 10,000 x g for 1 min at room temperature.

#### 6. Wash II

Place the HiBind® spin column (step 4) in the collection tube used in step 4 and add 750 µl of SPW Wash Buffer diluted with ethanol. Centrifuge at 10'000 x g for 1 min at room temperature. Discard the flow-through liquid and repeat step 5 with another 750 µl SPW Wash Buffer.

#### 7. Dry (Important, do not skip this step!)

Place the HiBind® spin column containing your DNA in the collection tube used in step 5 and centrifuge for 1 min at 10'000 x g to dry the column matrix. This step is essential to remove ethanol from the column.

#### 8. Elution

Place the spin column (step 6) into a fresh 1.5 ml microcentrifuge tube. Add 30 - 50 µl (depending on the desired final concentration of DNA) Elution Buffer directly to the binding matrix in the spin column and centrifuge for 1 min at 10'000 x g to elute DNA.

*This first elution represents approximately 80-90% of the bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.*

## peqGOLD Gel Extraction Protocol

### *Extraction of DNAs from PCR reaction*

#### 1. Agarose gel electrophoresis

Perform agarose gel/ethidium bromide electrophoresis to analyze PCR product.

#### 2. Load and Bind

Determine the volume of the PCR reaction, transfer to a clean 1.5 ml microfuge tube, add an equal volume of Buffer XP2 and vortex thoroughly.

*For PCR products <200 bp add up to 3 volumes of Buffer XP2; for fragments > 4 kb add 3 volumes Buffer XP2, followed by 1 volume aqua bidest.*

Apply the sample to an HiBind® DNA spin-column assembled in a clean 2 ml collection tube (provided) and centrifuge in a microcentrifuge at 10.000 x g for 1 min at room temperature. Discard the liquid.

Continue the preparation as described under step 6 of protocol 'Extraction of DNAs from Agrose gels'.

#### Yield and quality of DNA

Determine the absorption of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. One  $A_{260}$ -unit is about 50µg DNA/ml. The DNA concentration is calculated as follows:

$$DNA\ conc.\ (\mu g/ml) = Absorption_{260} \times 50 \times Dilution\ Factor$$

The ratio of  $A_{260/280}$  is an indication of nucleic acid purity. A value higher than 1.8 indicates > 90% nucleic acid. Typically, the majority of the DNA eluted is in monomeric supercoiled form, though concatamers may also be present.

*Alternatively, quantity (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations.*

#### Storage of DNA

DNA purified by the peqGOLD Gel Extraction Kit can be stored in TE Buffer or sterile, deionized water at -20 °C for years.

## Ordering information

For DNA from gels and PCR Reactions:

peqGOLD Gel Extraction Kit	12-2501-00	5 Preparations
<i>(C-Line)</i>	12-2501-01	50 Preparations
<i>(DNA from agarose gels)</i>	12-2501-02	200 Preparations
peqGOLD Gel Extraction Kit	12-2500-00	5 Preparations
<i>(S-Line)</i>	12-2500-01	50 Preparations
<i>(DNA from agarose gels)</i>	12-2500-02	200 Preparations
peqGOLD MicroSpin Gel Extraction Kit	12-6294-00	5 Preparations
<i>(DNA from agarose gels)</i>	12-6294-01	50 Preparations
	12-6294-02	200 Preparations
peqGOLD Cycle-Pure Kit	12-6493-00	5 Preparations
<i>(C-Line)</i>	12-6493-01	50 Preparations
<i>(DNA from PCR Products)</i>	12-6493-02	200 Preparations
peqGOLD Cycle-Pure Kit	12-6492-00	5 Preparations
<i>(S-Line)</i>	12-6492-01	50 Preparations
<i>(DNA from PCR Products)</i>	12-6492-02	200 Preparations
peqGOLD MicroSpin Cycle-Pure Kit	12-6293-00	5 Preparations
<i>(DNA from PCR Products)</i>	12-6293-01	50 Preparations
	12-6293-02	200 Preparations

## Troubleshooting Tips

Problem	Likely cause	Suggestion
Low DNA yields.	Too little Binding Buffer added to gel.	Volume of agarose gel slice was determined incorrectly. Add enough Binding Buffer as instructed.
	Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 55 °C to 65 °C and allow gel to melt completely.
	TAE running buffer was not fresh.	With overuse, TAE loses its buffering capacity and increases in pH. This raises the pH of the agarose/ DNA/ Binding Buffer solution which interferes with DNA binding to HiBind® matrix. Use freshly prepared TAE buffer for gel purification (and prevent contamination of isolated DNA in addition to improving yields).
Column clogged.	Agarose gel not completely dissolved in Binding Buffer.	Make sure water bath is set to 55 °C to 65 °C and allow gel to melt completely. For large agarose slices (>0.3 ml) it is recommended that the gel is diced into smaller fragments to aid melting.
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare Wash Buffer Concentrate with 100% ethanol as instructed above.
Optical densities do not agree with DNA yield on agarose gel.	Trace contaminants eluted from column increase $A_{260}$ .	Make sure to wash column as instructed in step 5. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantitation.
DNA floats out of well while loading agarose gel.	Ethanol not completely removed from column following wash steps.	Centrifuge column as instructed in step 6 to dry completely.