

# **peqGOLD Cycle Pure 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® 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. The Cycle-Pure Kit is a convenient system for fast and reliable purification of PCR products. The method uses HiBind® technology to recover DNA bands 50 bp-40 kb free of oligonucleotides, nucleotides, and polymerase in yields exceeding 80%. Binding conditions are adjusted by addition of a specially formulated buffer, and the sample is applied to a HiBind® DNA spin-column. Following a rapid wash step, DNA is eluted with deionized water (or low salt buffer) and ready for other applications. No organic extractions or alcohol precipitations means safe and rapid processing of multiple samples in parallel. The product is suitable for T-A ligations, PCR sequencing, restriction digestion, or various labelling reactions. In addition the kit can be used to purify DNA from any other enzymatic reaction.

peqGOLD Cycle-Pure Kits are available as S- or C-line columns (Safety-Line, Best.-Nr. 12-6492-xx or Classic Line, Best.-Nr. 12-6493-xx). S-line columns have a slender form and a tube-like outlet at the bottom, ensuring that all positions in centrifuges and vacuum manifolds can be occupied. Lids close the columns tightly and avoid cross-contamination. C-line columns have a bigger diameter and a flat bottom, allowing higher sample volumes to be processed. These columns possess no lid.

## Benefits

The peqGOLD Cycle-Pure Kit means:

- ! Speed - DNA recovery from enzymatic reactions <15 min
- ! Reliability - optimized buffers guarantee pure DNA
- ! Safety - No organic extractions
- ! Quality - purified DNA suitable for any application

## Binding Capacity

Each HiBind® DNA column can bind ~30 µg DNA.

## Kit Components

peqGOLD Cycle Pure Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-6493-00	12-6493-01	12-6493-02
<b>Components</b>			
HiBind <sup>®</sup> DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
Buffer XP1	1,5 ml	20 ml	80 ml
SPW Buffer Concentrate	5 ml	20 ml	3 x 20 ml
Elution buffer	1,5 ml	15 ml	60 ml
Instruction manual	1	1	1

## Storage and Stability

All peqGOLD Cycle-Pure Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22-25°C. Under cool ambient conditions crystals may form in Buffer XP1. Simply warm to 37°C to dissolve.

## Before Starting

SPW Buffer Concentrate must be diluted with absolute ethanol as follows:

Kit 12-6493-00	Add 20 ml ethanol to 5 ml wash buffer
Kit 12-6493-01	Add 80 ml ethanol to to 20 ml wash buffer
Kit 12-6493-02	Add 3 x 80 ml ethanol to 3 x 20 ml wash buffer

## peqGOLD Cycle-Pure Protocol

Please read this booklet thoroughly to ensure that you are familiar with the entire procedure. peqGOLD Kits are designed to be simple, fast, and reliable provided that all steps are followed diligently. All centrifugation steps must be performed at room temperature.

Materials Supplied By User:

- ! Microcentrifuge capable of at least 10,000 x g.
- ! Sterile 1.5 ml centrifuge tubes.
- ! Sterile deionized water (optional)
- ! Absolute (or 95%) ethanol

### 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, and add equal volume of Buffer XP1 and vortex thoroughly.

*For PCR products <200 bp add 3 volumes of Buffer XP1.*

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.

### 3. Wash

Wash the column by adding 750 µl of SPW Buffer diluted with absolute ethanol. Centrifuge at 10,000 x g for 1 min at room temperature. Discard liquid and repeat step 4.

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

Discard liquid and centrifuge the empty column for 1 min 10,000 x g to dry the column matrix. This is critical for good DNA yields.

### 5. Elution

Place column into a clean 1.5 ml microcentrifuge tube. Add 30-50 µl (depending on desired concentration of final product) Elution buffer or sterile deionized water directly onto the column matrix and centrifuge 1 min at 10,000 x g to elute DNA.

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

## Concentration of DNA

If required PCR products purified with peqGOLD Cycle-Pure Kit can be concentrated. Therefore add NaCl up to an end concentration of 0.1M and then add 2 volumes of ethanol. Vortex thoroughly and incubate the mixture for 10 min at  $-20^{\circ}\text{C}$ . Centrifuge at  $10,000 \times g$  for 15 min. Discard the supernatant, add  $700\mu\text{l}$  80% Ethanol and centrifuge again at  $10,000 \times g$  for 2 min. Discard the supernatant and air-dry the pellet. Solve the dry DNA in  $20\mu\text{l}$  sterile deionized water (or TE buffer).

## Yield and quality of DNA

Determine the absorbance of an appropriate dilution of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance } 260 \times 50 \times (\text{Dilution Factor})$$

$\mu\text{g/ml}$

Fragments greater than 500 bp in length can routinely be purified at >80% yield. Bands ranging from 50 bp to 500 bp give yields of 60%-90%. The ratio of (absorbance 260)/(absorbance 280) is an indication of nucleic acid purity. A value greater than 1.8 indicates > 90% nucleic acid. Alternatively, yield (as well as quality) can sometimes best be determined by agarose gel/ethidium bromide electrophoresis.

## Short Protocol For Experienced Users

1. Determine volume of reaction. Add 2-3 volumes of Buffer XP1 to PCR reaction.
2. Apply solution to HiBind<sup>®</sup> DNA column assembled in 2ml collection tube.
3. Centrifuge at maximum speed 1 min at room temperature. Discard liquid.
4. Wash column twice with  $750\mu\text{l}$  SPW Buffer diluted with ethanol.
5. Centrifuge empty column 1 min at max speed to dry.
6. Place column into clean 1.5 ml tube and elute DNA with  $30\text{-}50\mu\text{l}$  Elution buffer or sterile water. Centrifuge 1 min.

## Ordering Information

For DNA extraction from enzymatic reactions

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

## Troubleshooting Tips

Problem	Likely cause	Suggestion
Low DNA yields.	Too little Buffer XP1 added to sample.	Add XP1 Buffer as indicated.
No DNA eluted.	Wash Buffer Concentrate not diluted with absolute ethanol.	Prepare SPW Buffer Concentrate 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 3. 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 4 to dry before proceeding to elution step.