

# **peqGOLD Blood DNA Kit (Safety-Line)**

**– Instruction Manual –**

## Contents

Introduction .....	3
Principle.....	3
Kit Components .....	4
Storage and Stability.....	4
Binding Capacity .....	4
Before Starting.....	5
A. Blood DNA Protocol .....	6
B. Blood Protocol for 1 ml Samples.....	7
C. Blood Protocol for Dried Samples.....	8
D. Buffy Coat.....	9
Determination of Yield and Quality .....	9
Ordering Information .....	10
Troubleshooting Guide .....	11

## Introduction

The peqGOLD Blood DNA Kit provides a rapid and easy method for the isolation of up to 30 µg genomic DNA from up to 1 ml fresh, frozen, and anticoagulated whole blood. The method can also be used for preparation of genomic DNA from buffy coat, serum, and plasma. The kit allows single or multiple, simultaneous processing of samples in under 20 minutes. Normally, up to 1 ml of whole blood can be used in a single experiment. There is no need for phenol/chloroform extractions, and time-consuming steps such as CsCl gradient ultracentrifugation, and precipitation with isopropanol or ethanol, are eliminated.

DNA purified using the peqGOLD Blood DNA method is ready for applications such as PCR\*, Southern blotting, and restriction digestion.

peqGOLD Blood DNA Mini Kits I are available as S- or C-line columns (Safety-Line, # 12-3482-xx or Classic Line, Best.-Nr. 12-3492-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.

## Principle

peqGOLD Blood DNA Kits use the reversible nucleic acid-binding properties of HiBind® matrix, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows genomic DNA up to 60 kb to bind to the matrix. Samples are first lysed under denaturing conditions and then applied to the HiBind® spin columns to which DNA binds, while cellular debris, hemoglobin, and other proteins are effectively washed away. High quality DNA is finally eluted in sterile deionized water or low salt buffer.

## Kit Components

peqGOLD Blood DNA Mini Kit	5 Purifications	50 Purifications	200 Purifications
Product Number	12-3482-00	12-3482-01	12-3482-02
<b>Components</b>			
HiBind® DNA Columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer BL	5 ml	20 ml	60 ml
DNA Wash Buffer Concentrate	12 ml	40 ml	3 x 40 ml
HB Buffer	3 ml	30 ml	110 ml
Elution Buffer	2 ml	30 ml	100 ml
OB-Protease	3 mg	30 mg	120 mg
RNase A	30 µl	260 µl	1.15 ml
10 mM TE buffer	1.5 ml	1.5 ml	4 x 1.5 ml

## Storage and Stability

All components of the peqGOLD Blood DNA Kit, except the OB Protease should be stored at 22°C-25°C. Once reconstituted in water, OB Protease must be stored at -20°C. Under cool ambient conditions, a precipitate may form in the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BL at room temperature.

## Binding capacity

Each HiBind® column can bind approximately 30 µg DNA. Using greater than 1 ml whole blood or 250 µl buffy coat is not recommended.

## Before Starting

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

- ! Buffer BL contains a chaotropic salt. Use gloves and protective eyewear when handling this solution.
- ! Under cool ambient conditions, a precipitate may form in the Buffer BL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer BL at room temperature.
- ! DNA Wash Buffer is concentrated and has to be diluted with absolute ethanol as follows:

Kit 12-3482-00	Add 18 ml ethanol to 12 ml Washbuffer
Kit 12-3482-01	Add 60 ml ethanol to 40 ml Washbuffer
Kit 12-3482-02	Add 3 x 60 ml ethanol to 3 x 40 ml Washbuffer

Store diluted DNA Wash Buffer at room temperature.

- ! Reconstitute OB Protease in 150 µl (Kit 12-3482-00) or 1.5 ml (Kit 12-3482-01) or 6 ml (Kit 12-3482-02) TE buffer. Vortex vial briefly prior to use. We recommend that you aliquot and store vials at -20°C.
- ! All steps must be carried out at room temperature.

## peqGOLD Blood DNA Mini Protocol

### A. Blood

The procedure below has been optimized for use with FRESH or FROZEN blood samples 1 to 250  $\mu$ l in volume. Anticoagulated blood, or Buffy Coat can also be used. Larger samples (up to 1 ml) can be processed according to the modification outlined in protocol B. In addition,  $10^7$  leukocytes or cultured cells may be used with this procedure.

#### Materials Supplied by User

- ! Microcentrifuge and sterile 1.5 ml tubes and pipet tips
- ! Water bath - set to 70°C.
- ! Isopropanol - approximately 0.3 ml per sample
- ! 100% Ethanol - (for cultured cells and leukocytes)

### 1. Lysis

Add sample to a sterile microcentrifuge tube and bring the volume up to 250  $\mu$ l with 10 mM Tris-HCl, PBS, or Elution Buffer provided. Add one after the other 25  $\mu$ l OP Protease and 250  $\mu$ l of Buffer BL. Vortex 10 sec to mix thoroughly. Add 5  $\mu$ l RNase A solution and incubate sample at 70°C for 10 min. Briefly vortex the tube once during incubation.

### 2. Load and Bind

Add 260  $\mu$ l of isopropanol to lysate and mix. Assemble a HiBind® DNA spin column in a 2 ml collection tube (provided). Transfer the solution into the column and centrifuge at 8,000 x g for 1 min. Discard the collection tube and flow-through liquid.

For buffy coat, isolated leukocytes, and cultured cells yields will improve if 260  $\mu$ l absolute ethanol is used in place of isopropanol.

Place the column into a new 2 ml collection tube and add 500  $\mu$ l of HB buffer. Centrifuge at 8,000 x g for 30 sec. Discard the flow-through liquid.

### 3. Wash I

Place the column into a new 2 ml collection tube and add 600  $\mu$ l of DNA Wash Buffer. Centrifuge at 8,000 x g for 1 min. Discard the flow-through liquid.

*Note that DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol as indicated on the bottle and page 3. If refrigerated, the diluted wash buffer must be brought to room temperature before use.*

#### 4. Wash II

Repeat the washing step described in step 3 with 600 µl of Wash Buffer.

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

Using the same 2ml collection tube, centrifuge at maximum speed (10,000 x g) for 2 min to dry the column.

#### 6. Elution

Place the column into a sterile 1.5 ml microfuge tube and add 200 µl of preheated (70°C) Elution Buffer. Allow tubes to sit for 2 min at room temperature.

To elute DNA from the column, centrifuge at 8,000 x g for 1 min. Retain flow-through containing the DNA.

Place column into a second 1.5 ml tube and repeat elution step with another 200 µl of preheated Elution Buffer. Discard column.

*Note: Each elution typically yields 60%-70% of the DNA bound to the column. Thus two elution generally give >90%. However, increasing elution volume reduces the concentration of the final product.*

### B. Protocol for 1 ml of Blood

For processing blood samples 250 µl to 1 ml in volume, increase the volumes of OB Protease, Buffer BL, and isopropanol in proportion.

#### 1. Lysis

Use up to 1 ml whole blood, plasma, serum, or body fluids (or 50 million leukocytes or cultured cells in 1 ml PBS). Adjust the total volume using PBS to a multiple of 250 µl. Add 25 µl OB Protease stock solution per 250 µl of sample. Add 250 µl Buffer BL per 250 µl of sample. Mix thoroughly by vortexing. Incubate at 70°C for 10 min mixing once by vortexing.

#### 2. Load and Bind

Add 260 µl isopropanol per 250 µl of initial blood volume. Completely mix by vortexing. Apply 650 µl of the lysate to an HiBind® DNA column assembled in a 2 ml collection tube. Centrifuge 1 min at 8,000 x g and discard the flow-through. Repeat until the entire lysate has been applied to the column.

- Place HiBind® column into a clean 2 ml tube. Follow the basic peqGOLD Blood DNA Protocol A from step 3.

*It is not recommended to use > 1 ml blood per column as this can exceed the DNA binding capacity or clog the column thus reducing yield and quality.*

## C. Blood Protocol for dried blood samples

Dried blood samples on filter paper can be processed using the following method. You will require Buffer TL (12-TL-01) for this protocol. We recommend using OB Specimen Paper (OBP-01 and OBP-02) for spotting blood as this unique filter paper disintegrates when incubated in aqueous buffers and allows efficient recovery of DNA.

### 1. Homogenization and Lysis

Cut or punch out the blood spot from the filter paper. (Up to 200 µl blood can be used for each spot.) Tear or cut filter into small pieces and place into a microfuge tube. Add 250 µl Buffer TL and incubate at 95°C for 1-2 h. Vortex to mix every 20 min. Cool down the reaction mix to 60°C and add 25 µl OB protease solution (12-3492) and mix. Incubate 30min at 60°C with occasional mixing.

### 2. RNase Digest (optional)

Depending on the downstream Application a RNase digest might be necessary. If so add 5µl RNase A (25mg/ml) per 200µl dried Blood and incubate 2 min at room temperature.

### 3. Load and Bind

Centrifuge at 10,000 x g for 1 min at room temperature. Transfer the supernatant to a clean microfuge tube and add **ONE** volume of Buffer BL followed by **ONE** volume of isopropanol. Vortex thoroughly to mix. **Tip:** For example, if only 200 µl of clear supernatant is obtained, add 200 µl Buffer BL followed by 200 µl isopropanol.

- Add the mixture to a HiBind® DNA mini column assembled in a 2 ml collecting tube and proceed with the main Blood DNA protocol A from step 3.

*Blood spots from finger pricks usually contain no more than 50 µl blood and yield approximately 500 ng to 1 µg DNA. This is usually sufficient for PCR analysis. To obtain higher DNA concentrations, elute with 50 µl preheated Elution Buffer or TE and repeat with the first eluate.*

## D. Buffy Coat

The buffy coat fraction of whole blood is enriched with WBC, and usually gives at least 5-fold more DNA than the same volume of blood. To prepare buffy coat from fresh whole blood, simply centrifuge the sample at 3,000-4,000 x g for 10 min at room temperature. Three layers should be obtained with plasma in the upper layer, leucocytes in the middle layer (buffy coat), and erythrocytes in bottom layer. Carefully aspirate the plasma making sure not to disturb the layer of concentrated leukocytes. The buffy coat can be drawn off with a pipette and used directly in the peqGOLD Blood DNA Protocol, or frozen at  $-70^{\circ}\text{C}$  for storage.

## Determination of Yield and Quality

The total DNA yield can be determined by a spectrophotometer using deionized water, Tris-HCl buffer, or Elution Buffer as blank. Dilute the DNA in TE buffer and calculate concentration as:

$$[DNA] = (Absorbance_{260}) \times (0.05 \text{ :g/:l}) \times (Dilution \text{ factor})$$

Measuring absorbance at both 260 nm and at 280 nm can assess the quality of DNA. A ratio of (A<sub>260</sub>/A<sub>280</sub>) of 1.7-1.9 corresponds to 85%-95% purity.

Expected yields range from 4 µg to 12 µg DNA per 250 µl whole blood, depending on source of sample, its age, and the method of storage. Yields are generally 5-fold higher with Buffy Coat samples.

## Ordering information

peqGOLD Blood DNA Mini Kit (Classic – Line)	12-3492-00	5 Preparations
	12-3492-01	50 Preparations
	12-3492-02	200 Preparations
peqGOLD Blood DNA Mini Kit (Safety – Line)	12-3482-01	5 Preparations
	12-3482-02	50 Preparations
	12-3482-03	200 Preparations

## Troubleshooting Tips

Problem	Likely cause	Suggestion
Clogged Column.	Incomplete lysis	Add the correct volume of Buffer BL and incubate for specified time at 70°C. It may be necessary to extend incubation time by 10 min.
	Sample too large	If using more than 250 µl of blood, increase volumes of OB Protease, Buffer BL, and isopropanol. Pass aliquots of lysate through one column successively.
	Sample too viscous	Divide sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl.
Low DNA yield	clogged column	See above
	Poor elution	Repeat elution or increase elution volume Incubation of column at 70°C for 5 min with Elution Buffer may increase yields.
	Improper washing	Wash Buffer Concentrate must be diluted with absolute (100%) ethanol as specified before use.
	Buffy Coat used	With Buffy Coat samples, use absolute ethanol rather than isopropanol.
Low A260/A280 ratio	Extended centrifugation during elution step.	Resin from the column may be present in eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation — it will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer BL	Repeat the procedure, this time making sure to vortex the sample with Buffer BL immediately and completely.
	Hemoglobin remains on column	After application of sample to column, wash once with 300 µl Buffer AL.
No DNA eluted	Poor cell lysis due to improper mixing with Buffer BL.	Mix thoroughly with Buffer BL prior to loading HiBind™ column.
	Poor cell and/or protein lysis in Tissue Lysis Buffer.	Tissue sample must be cut or minced into small pieces. Increase incubation time at 65°C to ensure that tissue is completely lysed.
	Absolute ethanol not added to Buffer BL.	Before applying sample to column, an aliquot of Buffer BL/ethanol must be added. See protocol above.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Washing leaves colored residue in column	Incomplete lysis due to improper mixing with Buffer BL.	Buffer BL is viscous and the sample must be vortexed thoroughly.
	No ethanol added to Wash Buffer Concentrate.	Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Eluted material has red/brown color	Sample volume too large.	Reduce sample volume and follow directions
	Hemoglobin remains on column.	After applying sample, wash column once with 300 µl Buffer BL.