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User's Manual and Instructions

EZ-BLOOD DNA 96 KIT

Catalog# Z7040008-1 2 X 96 Purifications
Catalog# Z7040008-2 6 X 96 Purifications

INTRODUCTION

The EZ-Blood DNA 96 Kit is designed for the high-throughput purification of genomic DNA from whole blood in a 96-well plate format. No phenol-chloroform extraction or precipitation steps are involved. The sample addition and washing steps are performed using any 96-well compatible vacuum manifold, while the lysis of red blood cells (RBCs) and the final elution of the DNA product are performed using a table-top centrifuge.

The red blood cells (RBCs) in whole blood are first selectively lysed and the while blood cells are pelleted by centrifugation. The white blood cells are then lysed using an SDS-containing buffer and are treated with RNases and proteases, which are then heat-inactivated. A buffer containing the denaturant guanidine isothiocyanate and ethanol are then added to the samples, which are then added to the filter plates. This step facilitates the binding of DNA to the filter plate's silica membrane matrix. Under these conditions the DNA binds to the membrane while other contaminants are washed through. The EZ-Blood DNA 96 Kit's 96-well silica membrane plates have a standard Society for Biomolecular Screening (SBS) footprint and are compatible with a variety of automated liquid handling workstations, all 96-well-compatible vacuum manifolds and most, if not all, 96-well plate-compatible rotors. The plate is then washed to further remove protein, buffer components and other contaminants using two ethanol-containing wash buffers and the final genomic DNA product is eluted in TE. The final DNA product is essentially free of RNA and can be used directly for quantitative PCR and other downstream applications. Very small fragments of DNA including primers (<200 nucleotides) are not efficiently isolated using this kit.

KIT CONTENTS

Component	Contents per Kit
Cell Lysis buffer	1 X 10 ml
RNase solution	50 µl
Processing plates	2 X 96-well
Plate sealers	8 X 96-well plate covers
Protease solution	1 ml
Binding buffer	1 X 10 ml
Wash buffer 1 concentrate	1 X 70 ml (for 140 ml)
Wash buffer 2 concentrate	1 X 60 ml (for 300 ml)
Porous tape	4 X 96-well plate covers
Filter plates	2 X 96-well
Collection plates	2 X 96-well
TE	1 X 20 ml
96-well deep well plates	2
10X RBC lysis buffer	1 X 80 ml
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STORAGE CONDITIONS

The RNase and protease solutions should be kept at -20°C until required. Once the 10X RBC lysis buffer has been diluted with water it is stable for one week at room temperature. All other contents of the EZ-Blood 96 DNA Kit including the buffers should be stored at room temperature. The kit is stable for one year under these conditions.

SAFETY INFORMATION

The MSDS for this kit is available online at www.biochain.com.

TECHNICAL ASSISTANCE

Please refer any technical questions to TechSupport@biochain.com.

IMPORTANT NOTES BEFORE USING THE EZ-BLOOD DNA 96 KIT

Sample Size and Type

The EZ-Blood DNA 96 Kit can be used to isolate genomic DNA, plasmids and other DNA fragments bigger than ~200 bp from up to 200 µl of whole blood using a 96-well format. The use of greater volumes of blood may be associated with insufficient lysis of RBCs and clogging of the filter plates. DNA can be isolated quantitatively from less blood if required.

Buffer Concentrates

Wash buffers 1 and 2 are provided as concentrates that require the addition of 100% ethanol to them before use. The 10X RBC lysis buffer needs to be diluted 10-fold with distilled delonized water (1 part 10X buffer concentrate and 9 parts water) before use. The diluted RBC lysis buffer is stable for one week at room temperature.

Increasing Throughput

The protocol can be expedited considerably when the wash buffers are applied to the wells of the filter plate using a wash bottle to deliver the buffer rather than by pipetting.

Reagents and Equipment to be Supplied by the User

- Pipetteman (multichannel pipettors desirable) with sterile RNase-free tips
- Disposable gloves
- 100% ethanol
- Laboratory-grade adhesive tape
- Distilled deionized water
- Paper towels
- Sterile tubes or bottles for dilution of RBC lysis buffer concentrate
- Any 96-well plate-compatible vacuum manifold
- A vacuum source with a capacity of 18 liters/min. The use of a weak vacuum may reduce the RNA yield and purity
- A table-top centrifuge capable of providing 650g with rotors that can accommodate 96-well plates (including GH3.8, GH3.8A and J34.3)

EZ-Blood DNA 96 KIT PROTOCOL

Before starting: If crystals appear in the binding buffer then it should be warmed briefly at 37°C to solubalize it. The wash buffer 1 concentrate requires the addition of 70 ml of 100% ethanol before it can be used, while the wash buffer 2 concentrate requires that 240 ml of 100% ethanol is added to it before use. Both of the wash buffers are stable for one year after the addition of ethanol. Thaw the RNase and protease solutions to room temperature before beginning. The 10X RBC lysis buffer needs to be diluted 10-fold with distilled deionized water (1 part 10X buffer concentrate and 9 parts water) into a sterile tube or bottle (not provided with kit) before use. The diluted RBC lysis buffer is stable for one week at room temperature.

1. Transfer (up to) 200 µl of whole blood into the wells of the 96-well deep well plates. Add 1.8 ml of RBC lysis buffer (that has been diluted appropriately with water) per well. Pipette up and down twice to mix, seal the plate with a plate sealer and incubate at room temperature for 5 min. Spin the plate in a table top centrifuge for 5 min. at 400 g.

This kit is designed for the isolation of genomic DNA from fresh whole blood. Whole blood should be collected in the presence of an anticoagulant. EDTA is the preferred anticoagulant, but other anticoagulants such as citrate or heparin may be employed. For optimal results, blood samples should be processed within 4 hr. of collection.

2. Pour off the supernatant and resuspend the pellets into 2 ml RBC lysis buffer per well. Incubate and centrifuge as above.

The pellet is best resuspended by adding 1 ml of RBC lysis buffer, adjusting the pipetman to a volume of 0.9 ml, and pipetting up and down 10 times. Next another 1 ml of RBC lysis buffer should be added per well and the mixture pipetted up and down twice.

3. Pour off the supernatant. Immediately before use, mix 5 µl RNase solution per ml Cell lysis buffer required and add 50 µl of this mixture to each plate well. Transfer the viscous slurry from each well into the corresponding well of the processing plate and seal the plate using a plate sealer. Here you should set your pipettor to a larger volume than 50 µl (say 80 µl) and transfer the slurry in a single pipetting. Spin down the plate briefly (10 sec at 500g) and place the processing plate for 2 hr at 37°C in a thermocycler, oven or other incubator.

The yield of genomic DNA will be reduced and the interwell variation increased if this incubation time is reduced to 1 hr. The protocol can be stopped after this incubation by leaving the plate overnight at room temperature. The plate should be transferred to 4°C the next morning if the protocol cannot be continued at that time.

4. Spin down the processing plate briefly as above, remove and discard the plate sealer, and add 5 µl of protease solution directly to each well and seal the plate with a new plate sealer. Place the processing plate for 2 hr at 55°C, followed by 10 min at 95°C in a thermocycler, oven or other incubator.

The yield of genomic DNA will be reduced and the interwell variation increased if this incubation time is reduced to 1 hr. The protocol can be stopped after this incubation by leaving the plate overnight at room temperature. The plate should be transferred to 4°C the next morning if the protocol cannot be continued at that time.

5. Place the filter plate onto the vacuum manifold. Spin down the processing plate briefly as above, add 50 µl of Binding Buffer per well and pipette the mixture up and down twice. Next add 100 µl of 100% ethanol per well to the samples containing binding buffer, pipette the plate well contents up and down three times to mix it well and add the contents to the corresponding well of the filter plate. Turn on the vacuum pump for 30 seconds or until all of the sample material has been drawn through the wells of the filter plate. Then release the vacuum pressure from the plate assembly before turning off the vacuum.

If some of the plate wells will not receive any samples, first cover the wells that will not be used with ordinary laboratory adhesive tape (not supplied). This helps to increase the vacuum pressure to the wells that are employed and these covered wells can be used another day.

As with all 96- and 384-well applications, some small differences in efficiency may be experienced when the outer wells of the plate, and in particular the plate corner wells, are used. The inner wells of the plate should be employed first whenever possible when experiments are designed.

6. Wash the wells by adding 700 µl wash buffer 1 (which contains the added ethanol) per well and apply the vacuum as above.

7. Wash the wells twice by adding 700 µl wash buffer 2 (which contains the added ethanol) and apply the vacuum as above.

8. Remove the filter plate from the vacuum manifold and pat it down firmly on a stack of paper towels until no further liquid is released onto the paper towels. Return the filter plate to the vacuum manifold assembly and turn on the vacuum for 5 min. to completely dry the membrane.

The plate wells need to be dry in order to prevent alcohol carryover to the DNA product in the final elution step. Some vacuum manifolds may accomplish this in 3 min., while others may require 10 min. The use of a 5 min. filter plate drying step is sufficient for most users.

9. Remove the cover of the Place 96-well collection plate and place the filter plate on top of the collection plate. Add 50 µl of TE per well, preferably using a multichannel pipettor. Cover the plate with the porous tape, wait 1 min., and then centrifuge the filter plate/collection plate assembly (650g X 2 min.) to elute the final DNA product. Repeat the elution using 50 µl TE as above.

The use of one 50 µl elution rather than two elution steps will provide you with a slightly more concentrated DNA product, however the absolute yield of DNA will be reduced and the intrawell variation increased. The dead volume of the filter plate wells is approximately 20 µl, so one can expect to recover approximately 80 µl of genomic DNA product when two elution steps are used.

Kit Performance

Figure 1 shows the results of the analysis of double-stranded DNA levels isolated from various volumes of blood from a healthy male volunteer using this kit. Picogreen reagent (Invitrogen) was used to measure sample fluorescence- which is directly proportional to double-stranded DNA content. The relationship between sample fluorescence and blood volume was highly linear (r^2 = 0.9975). Additional analysis of the DNA using real-time PCR analysis of 18S rRNA DNA copies was also linear with an r^2 = 0.9750, with a

maximum well-to-well variation as assessed by CV = mean/SD X 100% = 2.8% (not shown).

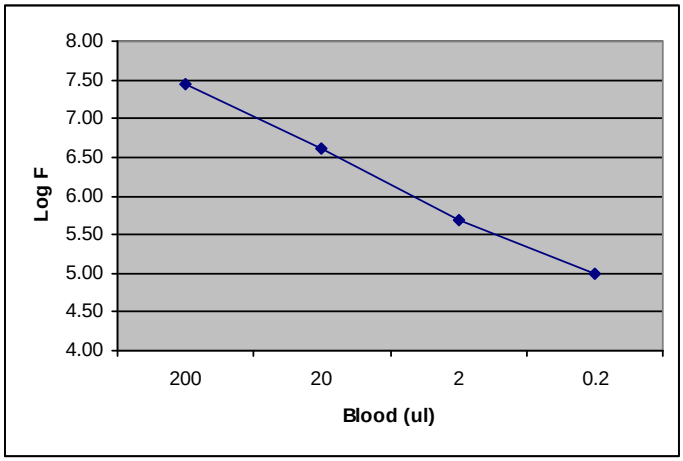

Figure 1. Picogreen fluorescence versus blood volume extracted for DNA isolated using the EZ-Blood DNA 96 Kit. Shown are the mean picogreen fluorescence observed for four replicates each of genomic DNA isolated at each indicated blood volume. The DNA was analyzed using picogreen reagent (Invitrogen) according to the manufacurer's protocol.

RELATED PRODUCTS

Component	Catalogue Number
Cell Lysis buffer	Z7040008-3
RNase solution	Z7040008-4
Collection plates	Z7040008-5
Binding buffer	Z7040008-6
Wash buffer 1 concentrate	Z7040008-7
Wash buffer 2 concentrate	Z7040008-8
Porous tape	Z7040008-9
96-well deep well plates	Z7040008-10
Plate sealers	Z7040008-11
10X RBC lysis buffer	Z7040008-12
Processing plates	Z7040008-13
Plate sealers	Z7040008-14
Protease solution	Z7040008-15
Filter plates	Z7040008-16
Collection plates	Z7040008-17
TE	Z7040008-18

TROUBLESHOOTING

Problem	Comments and Suggestions
Little or no DNA eluted	Remove all traces of supernatant before beginning. All buffers must be at room temperature. Ensure that vacuum draws all liquid through filter membrane at each step. Measure final elution volume - ensure adequate final elution from final centrifugation steps.
Filters clog	Too much DNA/cells used. Reduce sample size.
Filters tear/plates break	Reduce centrifugation speed.
DNA performs poorly	Ensure that that plate is completely dry and that remaining traces of ethanol have been removed before final elution step (increase drying time to 10 min).

EZ-BLOOD DNA 96 KIT
EXPERIENCED USERS MINIPROTOCOL

1. 200 µl whole blood & 1.8 ml RBC lysis buffer, mix, 5 min @ RT, 5 min @ 400g.
2. Pellet to 2 ml RBC lysis buffer, mix, 5min @ RT, 5 min @ 400 g.
3. 50 µl Cell lysis buffer containing RNase per well, transfer to processing plate, seal, incubate 2 hr at 37ºC.
4. Quick spin, 5 µl protease solution, seal, incubate 2 hr at 55ºC, 10 min. at 95ºC.
5. Quick spin, 50 µl binding buffer, mix, 100 µl EtOH, mix, add to filter plate, vacuum.
6. 700 µl wash buffer 1, vacuum.
7. 700 µl wash buffer 2, vacuum.
8. Repeat 700 µl wash buffer 2.
9. Pat filter plate on paper towels. Vacuum dry 5 min.
10. Place filter plate into collection plate, 50 µl of TE/well, cover with porous tape, incubate 1 min., centrifuge 650g X 2 min.
11. Repeat the elution using 50 µl TE.