For Research Use Only   Not for Diagnostic Use

EZ-DNA 96 Kit

Catalog# Z7040006-1 2 X 96 Purifications
Catalog# Z7040006-2 6 X 96 Purifications

INTRODUCTION

The EZ-DNA 96 Kit is designed for the high-throughput purification of genomic DNA from cultured cells in a 96-well plate format. No phenol-chloroform extraction or precipitation steps are involved. The kit can also be used to cleanup up to 96 DNA samples at a time. The sample addition and washing steps are performed using any 96-well compatible vacuum manifold, while the final elution of the DNA product is performed using a table-top centrifuge.

Cells are 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-DNA Kit 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	4 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
Instruction Manual	1

STORAGE CONDITIONS

The RNase and protease solutions should be kept at -20ºC until required. All other contents of the EZ-DNA 96 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-DNA 96 KIT

Sample Size and Type

The EZ-DNA 96 Kit can be used to isolate genomic DNA and other DNA fragments bigger than ~200 bp from between 50 to $5X10^5$ cultured cells using a 96-well format. DNA can still be isolated quantitatively from fewer cells if required, however the well-to-well variation and the extraction efficiency will be adversely affected. Similarly, DNA can be isolated from more cells if required, but a maximum of $5X10^5$ cultured cells were used during the testing of this product. Both adherent and suspension cultures can be utilized. Since confluent cultures of mammalian cells generally reach a maximum confluence of approximately $5X10^4$ cells/96-well, $1X10^5$ cells/48-well and $3X10^5$ cells/24-well plate, the kit can be used directly to isolate genomic DNA from a variety of original plate formats other than 96-well plates.

Wash Buffer

The EZ-DNA 96 Kit Wash Buffers 1 and 2 are provided as concentrates that require the addition of 100% ethanol to them before use.

Tissue Samples

Tissue samples can be used with the kit, however their performance has not been validated. Based on our experience with the kit we anticipate that 10 mg samples can be processed using the kit. The use of greater amounts of starting materials may lead to filter clogging, reduced DNA qualities and poor recoveries.

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 tips
- Disposable gloves
- 100% ethanol
- A 96-well thermocycler or adjustable-temperature oven or shaker
- Laboratory-grade adhesive tape
- Paper towels
- 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 DNA yield and purity
- A table-top centrifuge capable of providing 650 g with rotors that can accommodate 96-well plates (including GH3.8, GH3.8A and J34.3)

EZ-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.

1. Suspension cells should be aliquoted into the wells of a 96-well plate (not included) and centrifuged for 5 min. at 500g. Remove the supernatant completely by pipetting before proceeding with step 2.

Adherent cells grown in a 96-well plate can be used directly in step 2 after the complete removal of the media. Rinsing of the cells with PBS before continuing with the protocol may also be desirable.

2. 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 500 g) and place the processing plate for 2 hr at 37ºC 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 4ºC the next morning if the protocol cannot be continued at that time.

3. 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 55ºC, followed by 10 min at 95ºC 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 4ºC the next morning if the protocol cannot be continued at that time.

4. 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.

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

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

6. 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.

7. 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 avg/900g max 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 a TaqMan real-time PCR analysis of the levels of genomic DNA isolated when different numbers of cells were employed. Since the kit was so effective when even low quantities of cells were used, we chose to use the 18S rRNA gene, which is present at ~200 copies per haploid genome, for quantification of the genomic DNA levels. In this experiment we isolated genomic DNA from four replicates each of between 50-50,000 Hela cells. The levels of 18S rDNA copies was linear with an r^2 = 0.9956, with a maximum well-to-well variation as assessed by CV = mean/SD X 100% = 1.6%. The relationship between rDNA copies based on genomic equivalents as determined from a standard curve was also highly linear (r^2 = 0.9962), as was the relationship between cell number and DNA content as determined using picogreen reagent (Invitrogen; r^2 = 0.9964; not shown).

The 50,000 cell samples were also analyzed for 18S rRNA levels using TaqMan RT-PCR analysis. There was no difference in the observed Ct when PCR or RT-PCR was employed indicating that there was no detectable RNA contamination of the genomic DNA product.

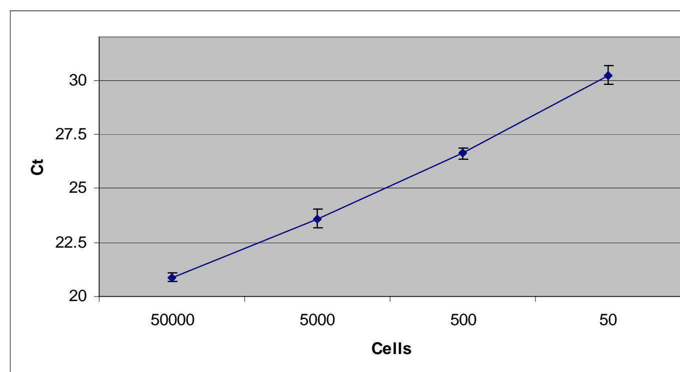

Figure 1. TaqMan PCR analysis of threshold cycle (C_t) versus cell number for DNA isolated using the EZ-DNA 96 Kit. Hela cells were trypsinized, counted in a hemocytometer, and then four replicates each of 50,000, 5,000, 500 and 50 were placed into the wells of a 96-well plate. The plate was centrifuged and the supernatants removed. The EZ-DNA 96 Kit protocol was then followed and the resulting DNA product was analyzed in an ABI Prism 7000 using 2X PCR master mix with TaqMan rRNA control reagents (Applied Biosystems, Inc.) under standard PCR conditions. Shown are the mean +/- SD C_t values observed.

RELATED PRODUCTS

Component	Catalogue Number
Cell Lysis buffer (10 ml)	Z7040006-3
RNase solution (50 µl)	Z7040006-4
Processing plates (2 plates)	Z7040006-5
Plate sealers (4 covers)	Z7040006-6
Protease solution (1 ml)	Z7040006-7
Binding buffer (10 ml)	Z7040006-8
Wash buffer 1 concentrate (70 ml, for 140 ml)	Z7040006-9
Wash buffer 2 concentrate (60 ml, for 300 ml)	Z7040006-10
Porous tape (4 covers)	Z7040006-11
Filter plates (2 plates)	Z7040006-12
Collection plates (2 plates)	Z7040006-13
TE (20 ml)	Z7040006-14

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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-DNA 96 KIT
EXPERIENCED USERS MINPROTOCOL

1. 50 µl Cell lysis buffer containing RNase per well, transfer to processing plate, seal, incubate 2 hr at 37ºC.

2. Quick spin, 5 µl protease solution, seal, incubate 2 hr at 55ºC, 10 min. at 95ºC.

3. Quick spin, 50 µl binding buffer, mix, 100 µl EtOH, mix, add to filter plate, vacuum.

4. 700 µl wash buffer 1, vacuum.

5. 700 µl wash buffer 2, vacuum.

6. Repeat 700 µl wash buffer 2.

7. Pat filter plate on paper towels. Vacuum dry 5 min.

8 Place filter plate into collection plate, 50 µl of TE/well, cover with porous tape, incubate 1 min., centrifuge 650--900g X 2 min.

9. Repeat the elution using 50 µl TE.