PROTOCOL & TECHNICAL BULLETIN





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***** KIT CONTENTS

50 RXN Kit (GEN0001S):

ITEM	CATALOG #	50 RXN
Genomic DNA Extraction Buffer	GEN0002	1 bottle/25ml
Wash Buffer Concentrate	GEN0004	1 bottle/12ml
NucleoPur [™] Spin Columns	GEN0006	50 columns
Collection Tubes	CT0050	50 tubes

250 RXN Kit (GEN0001L):

ITEM	CATALOG #	250 RXN
Genomic DNA Extraction Buffer	GEN0003	1 bottle/125ml
Wash Buffer Concentrate	GEN0005	2 bottles/48ml
NucleoPur [™] Spin Columns	GEN0007	250 columns
Collection Tubes	CT0250	250 tubes

PRECAUTIONS

THIS PRODUCT IS NOT INTENDED OR APPROVED FOR USE IN HUMANS OR VETERINARY ANIMALS. THE USE OF THIS PRODUCT IN A THERAPEUTIC SET-TING IS HAZARDOUS AND MAY RESULT IN ILLNESS, INJURY AND/OR DEATH.

Please read these instructions carefully before using this System.

The reagents in this system have been formulated and tested to work exclusively with the BenevBio Genomic DNA System. This system may not perform as described if any reagent or procedure is replaced and/or modified.

For research use only. Not for human or diagnostic use.

WARRANTY

BenevBio guarantees the performance of all products when used as directed for their intended purpose. Should any product fail to perform satisfactorily for any reason other than misuse, BenevBio's sole liability hereunder shall be limited to refund of the purchase price or, at the discretion of BenevBio, the replacement of all material that does not meet our specifications. BenevBio shall not be liable otherwise or for incidental or consequential damages including, but not limited to, the cost of handling. We reserve the right to change or modify any components to enhance the performance or design. The Buyer must give notice within thirty (30) days after receipt of material or shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

BenevBio CUSTOMER CARE INFORMATION

BenevBio 23263 Madero, Suite A Mission Viejo, California 92691 USA

Sales/Customer Care:	888-43-BENEV
Direct:	949-457-2222
Fax:	949-457-2221

Internet: www.benevbio.com

STORAGE AND STABILITY

The system will perform as specified if stored dry at room temperature (20-25 $^{\circ}$ C). Under these conditions the kit can be stored for up to 6 months without any decline in performance and/or quality.

ADDITIONAL ITEMS REQUIRED

- 1. Microcentrifuge capable of reaching 16,000 x g.
- 2. Adjustable pipettor.
- 3. Ethanol.
- 4. Microcentrifuge tubes.
- 5. A source of "UltraPure" water. Water used to elute samples must be deionized and free of trace organic contaminants.

Note: UltraPure water is available for purchase.

INTRODUCTION AND DESCRIPTION

The BenevBio spin-column based Genomic DNA System is a quick and easy method of extracting total genomic DNA from animal tails, fresh or frozen tissues and cells. The NucleoPur[™]columns bind DNA, while proteins and other contaminants are washed away. Purified DNA is obtained free of contaminants, dnases, and enzyme inhibitors. The procedure does not implement the use of phenol/ chloroform and can be completed outside the confines of a flow hood.

The kit requires mechanical homogenization of tissues as opposed to enzymatic digestion. This method is ideal for small, quick preparations that can be completed in as little as 20 minutes. Using mechanical homogenization is a quicker and more affordable method for extracting whole genomic DNA which can be used in downstream applications such as PCR, enzymatic digestions and Real Time QPCR.

PROTOCOL

NOTE: Be sure to add an appropriate volume of ethanol (95-100%) to the Wash Buffer Concentrate before use. See bottle label for volume.

Tissues:

1. Homogenize tissue thoroughly in 200ul of Genomic DNA Extraction Buffer.

(If homogenate is viscous add another 200ul Extraction Buffer)

2. Add 100ul of 95-100% ethanol to homogenized samples.

(Add 0.5 volumes of ethanol for every 1 volume Extraction Buffer)

- 3. Vortex samples for 10-15 seconds
- Add total sample to spin column and centrifuge for 1 min. at 8,000xg.
 If using a viscous tissue a higher level spin (up to 16,000xg) may be necessary to fully pass sample through the column and prevent clogging.
 - Discard flow-through from collection tube.
- 5. After spin add 400 ul of wash buffer to the columns and spin for 1 min at 8,000xg.
- Remove spin column and discard flow-through.
 A new collection tube may be used if desired.

PROTOCOL (cont.)

- 7. Add 500 ul of wash buffer to column and centrifuge for 2 min at 16,000xg.
- 8. Remove spin column and place in a 1.5 ml microcentrifuge tube.
- Add 35-50ul H2O to the column and let stand for 1 minute.
- 10. Spin down samples for 1 min @ 16,000 x g for final elution.
- 11. (OPTIONAL). Add another 35-50uls RNase-free water directly to the column matrix and spin at 16,000 x g for 1 minute or use the original eluate from step 10 to the NucleoPur column to achieve a higher final concentration.
- 13. Use samples immediately or store at -80C for future use.

TROUBLESHOOTING

Low DNA yield:

Using correct amount of starting material

It is important to use the correct amount of starting material. Certain tissues with high cellular mass (i.e. liver, spleen), no more than 15-20 mg of tissue should be used. This will help in obtaining the highest yields of quality DNA and avoid co-purification of RNA. For tail snips a maximum of a 1.0-1.5 cm piece should be used.

Insufficient homogenization If too much starting material is used there may insufficient homogenization. This will result in the column becoming clogged. This can be avoided by adding more buffer to the homogenate or using less starting material

Ethanol was not added to wash buffer Ethanol must be added to the Wash Buffer Concentrate. Add an appropriate volume of ethanol and repeat procedure.

TROUBLESHOOTING (cont)

Low DNA yield:

It is vital to completely dry the membrane on the final wash step. To ensure this, make sure to use extra care when removing the spin column from the collection tube. If there is wash buffer on the base of the column it will contaminate the final product. If there is evidence or concern about wash buffer still being on the column repeat the spin.

RELATED PRODUCTS

ITEM	CATALOG #	SIZE
PCR Purification System	PPS0001S	50 RXN
Gel DNA Extraction System	GDE0001S	50 RXN

ORDERING INFORMATION

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