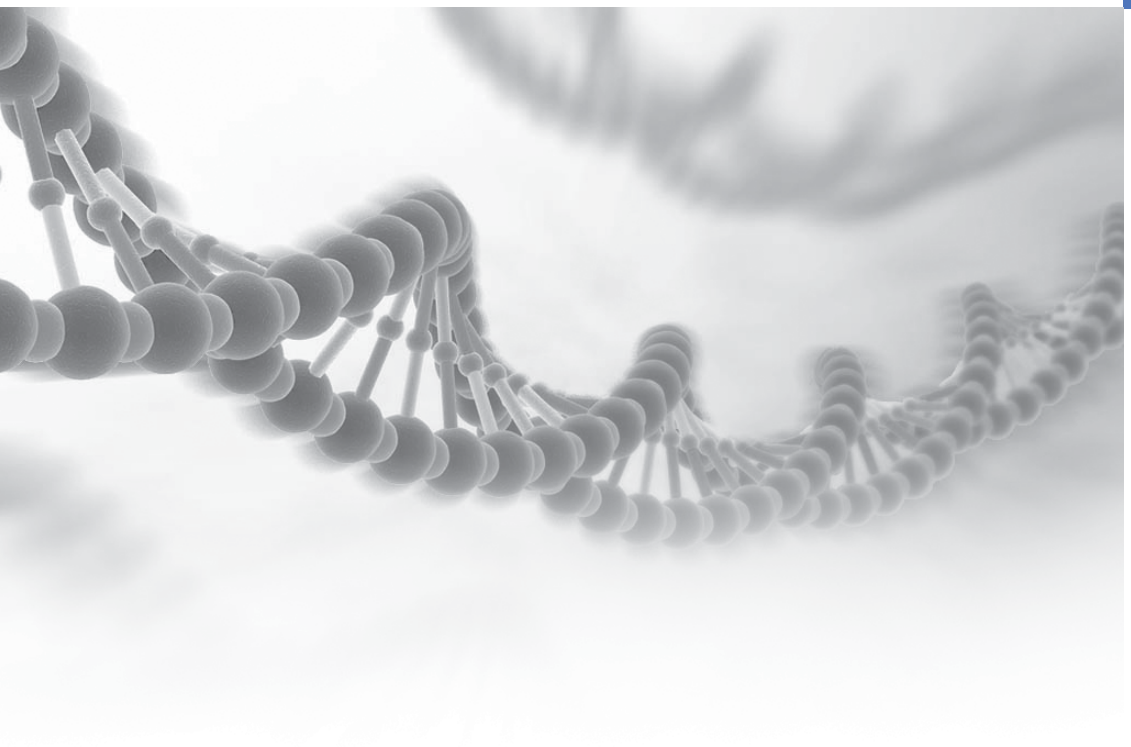
GENETIX BRAND

HANDBOOK

Nucleo-pore®



DNASure® PET Mini Kit

☐ **DNASure® PET Mini Kit**	NP-66405	50 Preps
☐ **DNASure® PET Mini Kit**	NP-66407	250 Preps

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Nucleo-pore®

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COMPONENTS

Kit contents

DNASure® PET Mini Kitt

Cat. No.	NP-66405	NP-66407
No of preps	50	250
Lysis Buffer PEL	20 ml	100 ml
Buffer PET1*	12 ml	60 ml
Buffer PET2*	3 ml	15 ml
Wash Buffer PEW	30 ml	2 x 75 ml
Wash Buffer PEW5 (Concentrate)*	2 x 7 ml	2 x 40 ml
Elution Buffer PEE	15 ml	75 ml
Proteinase K (lyophilized)*	30 mg	2 x 75 mg
Proteinase Buffer PB	1.8 ml	8 ml
DNASure® PETColumns	50	250
Collection Tubes (2 ml)	100	500
Labels for Lysis Buffer PET3	1	1
Handbook	1	1

* Please see “Preparation of Reagents”

Reagents, consumables and equipments not provided with the kit

- Absolute ethanol,
- Xylene (octane free)
- 1.5 ml microcentrifuge tubes for sample lysis and DNA elution
- Disposable tips
- Pipettes
- Centrifuge 1.5-2ml rotor adaptor
- Vortex mixer
- Heating-block or dry bath for incubation at 70 °C
- Equipment for sample disruption and homogenization (see section 2.5)
- Personal protection equipment (lab coat, gloves, goggles)

Safety instructions – risk and safety phrases

When working with chemicals always wear gloves lab coat and goggles and follow the safety instructions given in this section.

Buffer PET1
Contain Guanidinehydrochloride R & SPhrases: R22-36/38

Buffer PEW
Contain Guanidinehydrochloride and isopropanol R&S Phrases:R 10-22-36/38; S 7-16-25

Proteinase K
Contain lyophilized Proteinase K: R & S Phrases: R36/37/38-42; S 22-24-26-36/37

R 10 FlammableR 22 Harmful if swallowedR 36/37/38 Irritating to eyes, espiratory ystem and skinR 36/38 Irritating to eyes and skinR 42 May cause sensitization by inhalation; S 7 Keep container tightly losedS 16 Keep away from sources of ignition - No smoking!S 22 Do not breathe dustS 24 Avoid contact with the skinS 25 Avoid contact with he eyesS 26 In case of contact with eyes, rinse immediately with plenty of waterand seek medical adviceS 36/37 Wear suitable protective lothing and gloves

INTRODUCTION

Principle and Procedure

With the DNASure® PET Mini Kit method genomic DNA can be prepared from formalin (4-10%) fixed paraffin embeded tissue (fixation time 14-24 hrs). Lysis is achieved by incubation of the sample material in a proteinase K / SDS solution. Appropriate condition for binding of DNA to the silica membrane in the DNASure® PET Mini Kit Columns is achieved by the addition of chaotropic salts and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by subsequent washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Specifications of DNASure® PET Mini Kit

DNASure® PET Mini Kit is designed for the fast, small-scale preparation of highly pure gDNA from formalin fixed paraffin embedded tissue sections. The user friendly protocol allows easy deparaffinization of tissue and contaminant free DNA purification.

Sample material	Freshly cut tissue section of upto 10 μm thickness.
	Upto 10 section can be combined in one prep
Elution volume	20 – 100 µl
Format	Mini spin column

In addition to the standard method with recovery rate about 80% several modifications are possible to increase yield and concentration. Always

For High concentration: Perform one elution step with 60 % of the volume indicated in the individual protocol. Concentration of DNA will be ca. 30 % higher than with standard elution. The yield of eluted nucleic acid will be about 80 %.

For High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol, incubate for 3 min and centrifuge. Apply a second aliquot of elution buffer, incubate and centrifuge again. Thus, about 80%of bound nucleic acid is eluted in the standard elution volume at a high concentration.

Using elution buffer kept at ambient temperature may be used but this mayresult in a lower yield ofapproximately 20 % compared to elution with pre-heated elution buffer. Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal or higher than 8.

Note: For optimal performance of isolated DNA in downstream applications we recommend elution with the supplied elution buffer.

Preparation and Storage of Reagents

Precautions:

Buffers PET1, PET3, and PEW contain guanidine hydrochloride Always use personal protection equipments (PPE). All kit components can be stored at room temperature (18 – 25 °C) and are stable up to one year. During storage, especially at low temperatures, a white precipitate may form in Buffer PEL, PET1, or PET3. Such precipitates can be easily dissolved by incubating the bottle at 50 – 70 °C before use. Before starting any **DNASure® PET Mini Kit** protocol prepare the following:

Lysis Buffer PET3: Transfer the total contents of **Buffer PET1** to Buffer **PET2** and mix well. Place the labels for Lysis Buffer PET3 on the bottle. The resulting Lysis Buffer PET3 is stable for up to one year at room temperature.

Wash Buffer PEW5 (Concentrate)

Add 28ml and 160ml ethanol to each bottle of 7ml (NP-66405) and 40ml (NP-66407) of Wash Buffer PEW5 respectively. Store Wash Buffer PEW5 at room temperature (18 – 25 °C) for up to one year.

Reconstitution of Proteinase K

Add 1.35 ml and 3.35 ml of Proteinase Buffer PB to 30mg (NP-66405) and each 75mg (NP-66407) to lyophilized Proteinase K vials respectively. Proteinase K solution is stable at - 20 °C for up to 6 months.

Protocol for paraffin-embedded tissue

Things to do before starting

- Check that Buffer PET3, Buffer PEW5, and Proteinase K were prepared according to instructions in "Preparation & Storage of Reagents".
- Set dry bath or water bath to 37 °C and 56 °C.
- Before elution, preheat Elution Buffer PEE to 70 °C.
- Prepare small sections (up to 25 mg) from blocks of fixed, embedded tissue. Trim excess paraffin from the block before slicing and place the samples into microcentrifuge tubes with the help of tweezers.

Procedure

1. **Deparaffinization of tissue sections:**
   a. Add 1 ml n-octane or xylene to each tube containing tissue sections. Vortex vigorously and incubate at room temperature for about 30 min. Pulse vortex occasionally.
   b. Centrifuge at 11,000 x g for 3 min. Pipette off supernatant.
   c. Add 1 ml ethanol (96 – 100 %) to each tube. Close and mix by inverting several times.
   d. Centrifuge at 11,000 x g for 3 min. Pipette off supernatant.
   e. Repeat the ethanol washing step. Pipette off as much of the ethanol as possible.
   f. Incubate the open tube at 37 °C until the ethanol has evaporated (~ 15 min).

2. **Add 180 µl Buffer PEL and 25 µl Proteinase K solution. Vortex thoroughly. Incubate at 56 °C until achieved for 1 – 3 h. Vortex several times during incubation.** Be sure that the samples are completely covered with lysis solution. Incubation can be increased to overnight if samples are not completely lyzed.
   Note: Proteinase K and Buffer PEL may be premixed directly before use. Do not mix Buffer PEL and Proteinase K more than 10 – 15 min before addition to the sample .For RNA-free DNA an on column RNase digestion can be performed. By adding 20 µl RNase A (20 mg/ml) solution (not included) and incubate for an additional 5 min at room temperature.

3. **Add 200 µl Buffer PET3, vortex vigorously and incubate at 70 °C for 10 min. (vortex during incubation).**
   Note: If insoluble particles are visible, centrifuge for 5 min at high speed (e.g., 11,000 x g) and transfer the supernatant to a fresh microcentrifuge tube (not provided).

4. **Add 210 µl absolute ethanol to the sample and vortex vigorously.**
   Note: After addition of ethanol a stringy precipitate may appear. This will not affect the DNA isolation.Make sure all of the precipitate is loaded onto the column.

5. **For each sample, place one DNASure® PET Mini Kit Column into a Collection Tube. Apply the sample to the column without wetting the rim. Centrifuge for 1 min at 11,000 x g. Discard the flow-through and place the column back into the Collection Tube.** If the sample is not drawn completely through the matrix during centrifugation, repeat the centrifugation step at 11,000 x g. Discard flowthrough.

6. **Add 500 µl Buffer PEW. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.**

7. **Add 600 µl Buffer PEW5 to the column and centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the Collection Tube.**

8. **Centrifuge the column for 1 min at 11,000 x g. Residual ethanol is removed during this step. 11,000 x g**

9. **Place the DNASure® PET Mini Kit Column into a 1.5 ml microcentrifuge tube (not provided) and add 20-100 µl prewarmed Buffer PEE (70°C). Incubate at room temperature for 1 min. Centrifuge 1 min at 11,000 x g.**
   Note: Pre heat elution buffer PEE to 70 °C. For High yield: Perform two elution steps with the volume indicated in the individual protocol. About 80% of bound nucleic acid can be eluted.

TROUBLESHOOTING GUIDE

No or poor DNA yield

Possible cause
- Incomplete Lysis

Suggestions
- Sample not thoroughly homogenized and mixed with Buffer PEL /Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer PEL. Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible cause
- Reagents not applied properly

Suggestions
- Prepare Buffer PET3, Buffer PEW5, and Proteinase K solution according to instructions (see "Preparation and Storage of Reagents"). Add ethanol to the lysates before loading them onto the columns.

Possible cause
- Suboptimal elution of DNA from the column

Suggestions
- Preheat Buffer PEE to 70 °C before elution. Apply Buffer PEE directly onto the center of the silica membrane. Elution efficiencies decrease dramatically, if elution is achieved with buffers with a pH < 7.0. Use slightly alkaline elution buffers like Buffer PEE (pH 8.5). Especially when expecting high yields from large amounts of material, we recommend elution with 200 µl Buffer BE and incubation of the closed columns in dry bath at 70 °C for 5 min before centrifugation.

Poor DNA quality

Possible Cause
- Incomplete lysis

Suggestions
- Sample not thoroughly homogenized and mixed with Buffer PEL / Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer PEL.
  Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible Cause
- Reagents not applied properly

Suggestions
- Prepare Buffer PET3, Buffer PEW5, and Proteinase K solution according to instructions (see "Preparation & Storage of Reagents"). Add ethanol to the lysates before loading them on the columns.

RNA in sample

Possible Cause & Suggestion: If RNA-free DNA is desired, add 10 µl of RNase A solution (5 mg / ml; not supplied with the kit) before addition of Buffer PET3 and incubate at 37 °C for 5 min.

Columns Clogged

Possible cause
- Too much sample material used

Suggestions
- Do not use more sample material than recommended (25 mg for most tissue types). If insoluble material like bones or hair remains in the lysate, spin down the debris and transfer the clear supernatant to a fresh microcentrifuge tube before proceeding with addition of Buffer PET3 and ethanol.

Possible cause
- Incomplete lysis

Suggestions
- Sample not thoroughly homogenized and mixed with Buffer PEL / Proteinase K. The mixture has to be vortexed vigorously immediately after the addition of Buffer PEL. Decreased Proteinase K activity: Store dissolved Proteinase K at - 20 °C for 6 months.

Possible cause
- Reagents not applied properly

Suggestions
- Prepare Buffer PET3, Buffer PEW5, and Proteinase K solution according to instructions (see Preparation & storage of reagents). Add ethanol to the lysates before loading them on the columns.

Suboptimal performance of genomic DNA in enzymatic reactions

Possible Cause
- Carry-over of ethanol or salt

Suggestions
- Make sure to centrifuge ≥ 1 min at 11,000 x g in order to remove all of ethanolic Buffer PEW5 before eluting the DNA. If, for any reason, the level of Buffer PEW5 has reached the column outlet after drying, repeat the centrifugation. Do not chill Buffer PEW5 before use. Cold buffer will not remove salt effectively. Equilibrate Buffer PEW5 to room temperature before use.

Possible Cause
- Contamination of DNA with inhibitory substances

Suggestions
- Do not elute DNA with TE buffer. EDTA may inhibit enzymatic reactions. Repurify DNA and elute in Buffer PEE. If the A260 / A280 ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume Buffer PET3 plus 1 volume ethanol (96 – 100 %) to the eluate. Load the mixture onto a DNASure® PET Mini Kit® Tissue Column and proceed with protocol.

ORDERING INFORMATION

Description	Pack Size	Cat. No.
® DNASure Tissue Mini Kit	50 preps	NP-61305
® DNASure Plant Mini Kit	50 preps	NP-79105
® DNASure Plant Mini Kit	250 preps	NP-79107
® DNASure Plant Midi Kit	20 preps	NP-78153
® DNASure Plant Maxi Kit	10 preps	NP-78164
® DNASure Blood Mini Kit	50 preps	NP-61105
® DNASure Blood Mini Kit	250 preps	NP-61107
® DNASure Blood Midi Kit	20 preps	NP-61184
® DNASure Blood Maxi Kit	10 preps	NP-61193
® DNASure Blood FastPure Kit	50 preps	NP-62205
® DNASure Blood FastPure Kit	250 preps	NP-62207
® SureSpin Plasmid Mini Kit	50 preps	NP-37105
® SureSpin Plasmid Mini Kit	250 preps	NP-37107
® SureSpin Plasmid FastPrep Kit	50 preps	NP-47105
® SureSpin Plasmid FastPrep Kit	250 preps	NP-47107
® SureSpin Buffer Set*	1	37107-BS
® SurePrep Plasmid Mini Kit	20 preps	NP-15123
® SurePrep Plasmid Mini Kit	100 preps	NP-15125
® SurePrep Plasmid Midi Kit	20 preps	NP-15143
® SurePrep Plasmid Midi Kit	100 preps	NP-15145
® SurePrep Plasmid Maxi Kit	10 preps	NP-15161
® SurePrep Plasmid Maxi Kit	25 preps	NP-15162
® SurePrep Plasmid Mega Kit	5 preps	NP-15183
® SurePrep Plasmid Giga Kit	5 preps	NP-15191

***SureSpin® Buffer Set**

For the isolation of low-copy plasmids, buffers PA1, PA2, PA3, RNase A, sufficient for 300 preps

ORDERING INFORMATION

Description	Pack Size	Cat. No.
SurePrep® Buffer Set**	1	15143-BS
SurePrep® Plasmid Endofree Maxi Kit	10 preps	NP-15363
SurePrep Plasmid Endofree Mega Kit	5 preps	NP-15365
SurePrep® Plasmid Endofree Giga Kit	5 preps	NP-15367
SureSpin® 96 PCR Kit	4x96	NP-38151
SureTrap® Gel Extraction Kit	50 preps	NP-38705
SureTrap® Gel Extraction Kit	250 preps	NP-38707
SureTrap® PCR Cleanup Kit	50 preps	NP-38105
SureTrap® PCR Cleanup Kit	250 preps	NP-38107
SureExtract® Spin PCR/Gel Extraction Kit	50 preps	NP-36105
SureExtract® Spin PCR/Gel Extraction Kit	250 preps	NP-36107
SureSEQ® Cleanup Kit	50 preps	NP-73205
RNASure® Mini Kit	50 preps	NP-84105
RNASure® Mini Kit	250 preps	NP-84107
RNASure® Plant Kit	50 preps	NP-84905
RNASure® Plant Kit	250 preps	NP-84907
miRNASure® Mini Kit	50 preps	NP-71002
SureTrap® mRNA Mini Kit	12 preps	NP-80033
SureTrap® mRNA Midi Kit	12 preps	NP-80043
RNASure® Virus Kit	50 preps	NP-67705
RNASure® Virus Kit	250 preps	NP-67707

****SureSpin® Buffer Set**

For isolation of low-copy plasmids, cosmids, BACs, PACs, and P1 constructs, only applicable with SurePrep® Plasmid kits, sufficient for 10 SurePrep Maxi Columns (Maxi preps), 20 SurePrep® Midi Columns (Midi preps), set incl. RNase A

ORDERING INFORMATION

Description	Pack Size	Cat. No.
Nucleo-pore® Stool DNA Mini Kit	50	NP-7011D
Nucleo-pore® gRNA Blood Kit	50	NP-0201R
Nucleo-pore® gDNA Urine Kit	20	NP-6030D
Nucleo-pore® Yeast Transformation Kit	120	NP-1002T
Nucleo-pore® DNA Methylation Kit	50	NP-6006D
Nucleo-pore® gDNA Clean-up Kit	200	NP-4304D
Nucleo-pore® Bisulphite DNA Clean-up Kit	50	NP-5205D
Nucleo-pore® gDNA Fungal/Bacterial Kit	50	NP-7006D

Product Warranty

DNASure® PET Mini Kit components are intended for research purposes only. They are suitable for *in vitro* uses only. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Genetix will replace it free of charge or refund the purchase price. Genetix reserve the right to change, alter, or modify any product to enhance its performance and design. It is the responsibility of the user to verify the use of the DNASure® PET Mini Kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism. No claim or representation is intended for its use to identify any specific organism or for clinical or therapeutic use.

Genetix does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded), or out of accident or improper or abnormal use of this product.

In accordance with Genetix ISO-certified Quality Management System, each lot of DNASure® PET Mini Kit is tested against predetermined specifications to ensure consistent product quality.

In no event shall Genetix be liable for claims for any other damages, whether direct, indirect, incidental, compensatory, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Genetix products to perform in accordance with the stated specifications.

Product claims are subject to change. Therefore please contact our Technical Support Department for updated information on Genetix products.

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