GENETIX BRAND

HANDBOOK

Nucleo-pore™

PCR Clean-up / Gel Extraction

☐ **SureTrap® Gel Extraction Kit**	NP-38705	50 Preps
☐ **SureTrap® Gel Extraction Kit**	NP-38707	250 Preps
☐ **SureTrap® PCR Clean-up Kit**	NP-38105	50 Preps
☐ **SureTrap® PCR Clean-up Kit**	NP-38107	250 Preps

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Nucleo-pore™

Contents

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KIT CONTENTS

SureTrap® PCR Cleanup

Catalog No.	NP-38105	NP-38107
No of preps	50	250
SureTrap® PCR Clean-up Suspension (GCR)	500µl	2.5ml
Buffer SET2	50ml	5x50ml
Wash Buffer SET3 (Concentrate)*	10ml	50ml
Elution Buffer SEB	6ml	30ml
Handbook	1	1

SureTrap® Gel Extraction

Catalog No.	NP-38705	NP-38707
No of preps	50	250
SureTrap® Gel Extraction Suspension (GXR)	500µl	2,5ml
Buffer SET1	25ml	2x60ml
Buffer SET2	50ml	5x50ml
Wash Buffer SET3 (Concentrate)*	10ml	50ml
Elution Buffer SEB	6ml	30ml
Handbook	1	1

* Please see “Preparation of Reagents”

Reagents, consumables and equipment not provided with the kit

- 96 – 100 % ethanol
- 1.5 ml microcentrifuge tubes
- Centrifuge for microcentrifuge tubes
- Manual pipettes and disposable tips
- Vortex mixer
- Heating-block
- Personal protection equipment (lab coat, gloves, goggles)

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs).

Buffers SET1 and SET2 contain sodium perchlorate, which can be explosive if mixed with combustible material.

Buffer SET1
Contain sodium perchlorate: R& S phrases: R9-22, S13-27

Buffer SET2
Contain sodium perchlorate: R& S phrases: R9-22, S13-27
R9: Explosive when mixed with combustible material, R22: Harmful if swallowed, S13: Keep away from food, drink and animal foodstuffs, S27: Take off immediately all contaminated clothing.

INTRODUCTION

Principle and Procedure

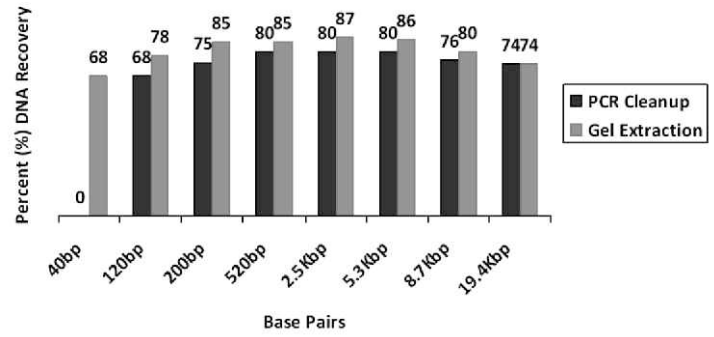
SureTrap® PCR clean-up / Gel Extraction method utilizes pre-optimized silica particles based DNA extraction from PCr reactions or kbp agarose gels. DNA fragments upto 20bp-20 Kbp can be purified from agarose gel and 120bp to 20Kbp from PCR reaction with varying recovery rates. All non nucleic acid impurities such as agarose, proteins, salts, ethidium bromide are efficiently removed during subsequent wash steps.

The pure DNA is suitable for most downstream applications like; sequencing, ligation, labeling, restriction digestion etc.

Specifications of SureTrap® PCR Cleanup / Gel Extraction Kits

The SureTrap® PCR Cleanup kit is designed for direct purification of PCR products. The SureTrap® Gel Extraction kit is designed for the purification of DNA from TAE / TBE agarose gels. In contrast to the SureTrap® Gel Extraction matrix, the SureTrap® PCR Cleanup matrix will not bind DNA fragments <100 bp due to a larger pore size of the silica matrix. Standard as well as low melting agarose gels can be used.

The prepared DNA fragments can be used directly in applications like automated fluorescent DNA sequencing, PCR, or any kind of enzymatic manipulation.



Graph showing DNA Recovery (%) of different fragment sizes using SureTrap® PCR Cleanup/Gel Extraction Kit

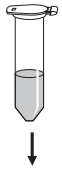
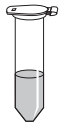
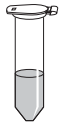
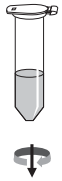
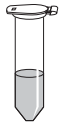
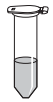
Preparation of reagents

Buffers SET1 and SET2 contain chaotropic salts. Wear gloves and goggles. The SureTrap® PCR Cleanup / Gel Extraction kits should be stored at room temperature and are stable for up to one year.

Wash Buffer SET3:

Reconstitute Wash Buffer SET3 by adding 40ml and 200ml ethanol (96-100%) respectively to 50 and 250 reaction kits (SureTrap® PCR Cleanup and SureTrap® Gel Extraction Kits) and mark the bottle "ethanol added".

PCR Clean-up / Gel Extraction

	SureTrap® PCR Clean-up		SureTrap® Gel Extraction
SureTrap®:Gel Excise DNA fragment Solubilize gel slice	4 volume of buffer SET2 1 volume of sample		300 µl Buffer SET1 100 mg gel
SureTrap®:PCR Adjust binding conditions			
Binding	10 µl PCR cleanup suspension 100 µl Sample RT, 10 min		4 µl PCR gel extraction suspension, 1 µg DNA 50 °C, 5 – 10 min
	10,000 x g, 30 sec		10,000 x g, 30 sec
Washing	400 µl Buffer SET2 10,000xg, 30 sec 400 µl Buffer SET3 10,000xg, 30 sec 400 µl Buffer SET3		500 µl Buffer SET2 10,000xg, 30 sec 500 µl Buffer SET3 10,000xg, 30 sec 500 µl Buffer SET3
	10,000 x g, 30 sec		10,000 x g, 30 sec
Drying	RT or 37°C 10 – 15 min		RT or 37°C 10 – 15 min
Elution	25 – 50 µl NE RT, 10-15 min		25 – 50 µl NE RT, 10-15 min
	10,000 x g, 30 sec		10,000 x g, 30 sec
Pure DNA			

Protocol for direct purification of PCR products using SureTrap® PCR Cleanup kit

Things to do before starting:

- Check if Wash Buffer SET3 was prepared as per instructions

Procedure

1. ***Add 4 volumes of Buffer SET2 to 1 volume of sample***
   For example 100µl of PCR reaction mixtures add 400 µl of Buffer SET 2 . For reactions mixture less than 100 µl adjust the volume to 100 µl using TE (pH 7.5). For reaction mixture more than 100 µl, volume should be increased proportionately.

2. ***Add 10 µl of SureTrap® PCR Cleanup Suspension (GCR) to each 100 µl of reaction mixture. Incubate the mixture for 10 min at room temperature (18-25°C). Pulse vortex. Centrifuge the sample at 10,000 x g for 30 s and discard the supernatant.***

3. ***Add 400 µl Buffer SET2 to the pelleted silica matrix and vortex for resuspension of the pellet. Centrifuge for 30 s at 10,000 x g and remove the supernatant completely.***

4. ***Add 400 µl Buffer SET3 to the pelleted silica matrix and vortex. Centrifuge for 30 s at 10,000 x g and remove the supernatant completely.***

5. ***Add 400 µl Buffer SET3 to the pelleted silica matrix and vortex briefly. Centrifuge for 30 s at 10,000 x g. Remove the supernatant and centrifuge the pellet again briefly. Remove residual Buffer SET3 completely.***

6. ***Dry the pelleted silica matrix at room temperature or at 37 °C for 10 – 15 min.***
   Sample should not be completely dried as over-dried pellets may lead to lower recovery rate.

7. ***Add 25 - 50 µl Buffer SEB to the silica matrix. Resuspend the pellet by vortexing. Incubate at room temperature for 10 - 15 min. Pulse vortex the mixture during incubation. Centrifuge at 10,000 x g for 30 s and transfer the DNA containing supernatant to a clean tube (not provided).***

   Optional: Repeat step 7 to increase the yield by ~10 %.
   Note: Yield of larger fragments (> 5 – 20 kbp) can be increased by performing the incubation at 55 °C.

Protocol for DNA extraction from agarose gels using SureTrap® Gel Extraction

Things to do before starting:

- Check if Wash Buffer SET3 was prepared as per instructions.
- Set heating block to 50 °C.

Procedure

1. ***Take a clean scalpel and cut the DNA fragment from agarose gel, ensuring minimum gel volume is taken. Weigh the gel size and transfer it in a clean micro centrifuge tube (not provided). Add three volumes of buffer SET 1 to one volume (in mg) of agarose gel .***
   Example, for 100 µg of agarose gel add 300 µl of Buffer SET 1
   Note: For gels containing > 2 % agarose, double the volume of Buffer SET1. For gel slice >100 mg, volume of Buffer SET1 must be increased proportionally.

2. ***Vortex Sure Trap® Gel Extraction Suspension (GXR). Add 4 µl of GXR to every 1 µg of DNA (Minimum of 10 µl of GXR is required to proceed). Incubate at 50°C till the gel slice is completely dissolved. Vortex and centrifuge for 30 sec at 10,000 X g. discard the supernatant.***

3. ***Add 500 µl Buffer SET2 to the pelleted silica matrix and vortex briefly for resuspension of the pellet. Centrifuge for 30 s at 10,000 x g and remove the supernatant completely.***

4. ***Add 500 µl Buffer SET3 to the pelleted silica matrix and vortex briefly. Centrifuge for 30 s at 10,000 x g and remove the supernatant completely.***

5. ***Add 500 µl Buffer SET3 to the pelleted silica matrix and vortex briefly. Centrifuge for 30 s at 10,000 x g. Remove the supernatant and centrifuge the pellet again briefly. Remove residual Buffer SET3 completely.***

6. ***Dry the pelleted silica matrix at room temperature or at 37 °C for 10 – 15 min.***
   Do not over dry the sample

7. ***Resuspend the pellet in 25-50 µl buffer SEB followed by pulse vortexing. Incubate at room temperature for 10 – 15 min. Pulse vortex the mixture 2 – 3 times during incubation. Centrifuge at 10,000 x g for 30 s and transfer the DNA containing supernatant to a clean tube (not provided).***

Supplementary protocol for concentration and removal of enzymes.

Things to do Before starting:

- Check if Wash Buffer SET3 was prepared as per instructions.

Procedure

1. ***Add 4 volumes Buffer SET2 to 1 volume of DNA containing sample (e.g., 400 µl Buffer SET2 and 100 µl reaction mixture).*** Vortex the SureTrap® PCR Cleanup / Gel Extraction Suspension thoroughly until a homogeneous mixture results.

2. ***For each µg of DNA add 4 µl of silica matrix (min 10 µl). Incubate the mixture for 10 min at room temperature and vortex briefly every 2 – 3 min. Centrifuge for 30 s at 10,000 x g and discard supernatan***t.
   Note: Be aware of the SureTrap® Gel Extraction Suspension binding fragments down to 20 bp.

   Continue with step 3 of SureTrap® PCR Cleanup protocol.

TROUBLESHOOTING GUIDE

Incomplete lysis of agarose slices

Possible cause
- High concentration of agarose

Suggestion(s)
- Use doubled volumes of Buffer SET1 for highly concentrated agarose gels.

Possible cause
- Wrong buffer

Suggestion(s)
- Buffer SET2 cannot be used for gel dissolution.

Possible cause
- Time and temperature

Suggestion(s)
- Check incubation temperature. Depending on the weight of gel slice, incubation can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Heavy weight gel slices may be quenched or crushed before addition of Buffer SET1.

No DNA yield

Possible cause
- Reagents not applied properly

Suggestion(s)
- Add indicated volume of 96 – 100% ethanol to Wash Buffer SET3 Concentrate and mix well before use.

Possible cause
- Insufficient drying of the SureTrap® PCR Cleanup / Gel Extraction silica matrix

Suggestion(s)
- Ethanolic Wash Buffer SET3 has to be removed quantitatively before elution. Prolong the drying time up to 30 min. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots).

Possible cause
- Isolation of large DNA fragments

Suggestion(s)
- Add room-temperature Elution Buffer SEB and incubate at 55 °C for 10 – 15 min.

Suboptimal performance of DNA in sequencing reactions

Possible cause

- Carry-over of ethanol/ethanolic Buffer SET3

Suggestion(s)

- Make sure to dry the silica matrix in order to achieve complete removal of ethanolic Buffer SET3 after the washing step. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Buffers other than Buffer SEB, for example TE buffer (Tris / EDTA), were used for elution of DNA. Note: EDTA may inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Buffer SEB or water.

Possible cause

- Not enough DNA used for sequencing reaction

Suggestion(s)

- Quantitate DNA by agarose gel electrophoresis before setting up sequencing reactions.

Possible cause

- SureTrap® PCR Cleanup or SureTrap® Gel Extraction particles were not removed quantitatively

Suggestion(s)

- Centrifuge the eluate again and transfer the supernatant to a new tube.

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

Product Warranty

SureTrap® PCR Cleanup/Gel Extraction Kits 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 SureTrap® PCR Cleanup/Gel Extraction Kits 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 SureTrap® PCR Cleanup/Gel Extraction Kits 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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