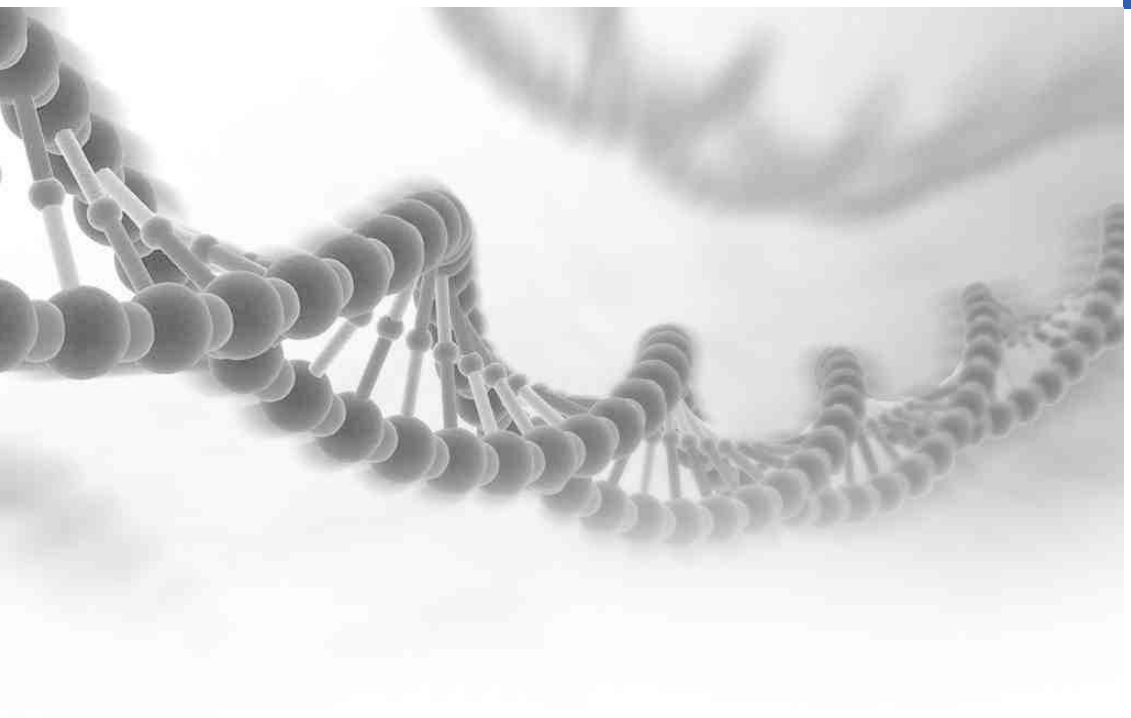
GENETIX BRAND

HANDBOOK

Nucleo-pore®



PCR Clean-up Gel Extraction

☐	**SureExtract® Spin PCR Clean-up/ Gel Extraction Kit**	NP-36105	50 Preps
☐	**SureExtract® Spin PCR Clean-up/ Gel Extraction Kit**	NP-36107	250 Preps

V 2.12

genetix Biotech Asia Pvt. Ltd.

Genetix Biotech Asia Pvt. Ltd.
71/1, First Floor, Shivaji Marg, Najafgarh Road, New Delhi - 110015
Phone : +91-11-45027000 ■ Fax : +91-11-25419631
E-mail : info@genetixbiotech.com ■ www.genetixbiotech.com

www.genetixbiotech.com

Nucleo-pore®

Table of Contents

*****

COMPONENTS

Kit Contents

SureExtract® Spin PCR/Gel Extraction Kit

Cat. No.	NP-36105	NP-36107
No of preps	50 preps	250 preps
Binding Buffer SET	2 x 25 ml	2 x 120 ml
Wash Buffer SET3 (Concentrate)*	2 x 10 ml	2 x 50 ml
Elution Buffer SEB	15 ml	50 ml
SureExtract® Spin Columns	50	250
Collection Tubes (2 ml)	50	250
Handbook	1	1

Reagents, consumables and equipments not provided with the kit

- 96 – 100% ethanol
- 1.5 ml microcentrifuge tubes
- Disposable pipette tips
- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Heating block
- Vortex mixer

SAFETY INSTRUCTIONS

When working with chemicals, always wear a suitable labcoat, disposable gloves and protective goggles. For more information please consult the appropriate MSDS.

Buffer SET
Contains Guanidine thiocynate
R & S phrases
R 20/21/22 Harmful by inhalation, in contact with skin, and if swallowed; S 13 Keep away from food, drink, and animal feedstuffs

INTRODUCTION

Principle and Procedure

The SureExtract® Spin PCR/Gel Extraction Kit is based on silica membrane technology. With its unique buffer system, DNA is efficiently purified from PCR products or agarose gels. DNA binds to a silica membrane in the presence of chaotropic saltpresent in Buffer SET, which is further loaded directly onto SureExtract® Spin Columns. Contaminations like salts and soluble macromolecular components are removed as flowthrough in a simple washing step with Wash Buffer SET3 containing ethanol. Pure DNA is finally eluted using Elution Buffer SEB

Specifications of SureExtract® Spin PCR/Gel Extraction Kit

The SureExtract® Spin PCR/Gel Extraction Combo Kit is designed for the direct purification of PCR products and for the purification of DNA from TAE/TBE agarose gels. DNA purified by the SureExtract® Spin PCR/Gel Extraction kit is significantly concentrated than DNA purified by other methods The unique buffer formulation of SureExtract® Spin PCR/Gel Extraction Kit enables complete removal of primers or unincorpoarated nucleotides from PCR reactions whereas, small DNA fragments remain bound to silica membrane column. Pure DNA is finally eluted using Elution Buffer SEB. With this kit, DNA fragments from PCR reaction buffers rich in various detergents can also be purified with high recovery. The adsorption of nucleic acids on silica membrane is pH dependent. It is recommended to use TAE standard gels or reaction mixtures between pH 6 and 8. The kit is recommended to use with regular as well as low melting agaroses.

The purified DNA fragments is ready for downstream applications like automated fluorescent DNA sequencing, labeling, hybridization, in vitro transcription, transformation, PCR, PCR-RFLP, or any other enzymatic reaction.

Binding capacity of SureExtract® Spin PCR/Gel Extraction kit is 15ug. The DNA can be eluted between 15 and 50ul of SEB

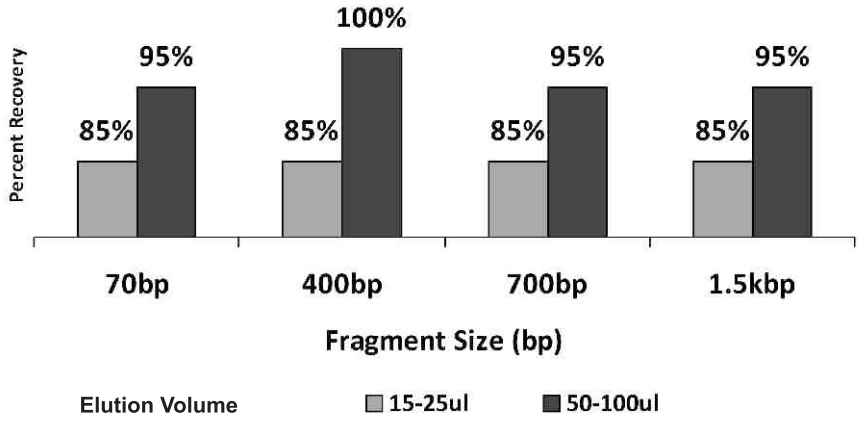
Dilution of Buffer SET for removal of different fragment sizes

SureExtract® Spin PCR/Gel Extraction Kit is designed to remove even traces of unused primers, unincorporated dNTPs, and purify PCR products down to 65 bp. If required, dsDNA of more than 65 bp can also be removed by lowering the binding efficiency for small fragments which is achieved by diluting an aliquot of Buffer SET with sterile water and then proceeding with the standard protocol.

Dilution of Buffer SET in a certain range lowers down the binding efficiency for small fragments without compromising the recovery of larger PCR products. Dilution ratio depends on the fragment size to be purified and the PCR buffer system that was used in amplification. Some reaction buffers contain detergents like Tween or betaine to lower down the Tm of the DNA template (especially in PCR buffers for high fidelity or long range PCR). They further lower down the binding efficiency of DNA to the silica membrane.

For removal of fragments up to 100bp from PCR products amplified using regular PCR buffer, 3 to 5 volumes of water to 1 volume of Buffer SET is recommended, whereas, in case of PCR buffer containing additives, adding 1 to 3 volumes of water to 1 volume of Buffer SET is recommended. Usually a dilution with 5 volumes of water is sufficient to eliminate even larger unwanted primer-dimer fragments while purifying the 165 bp fragment with recovery of > 90 %. Preheat Elution Buffer SEB for 1 – 2 min before elution.

DNA recovery with SureExtract® PCR/Gel Extraction Kit



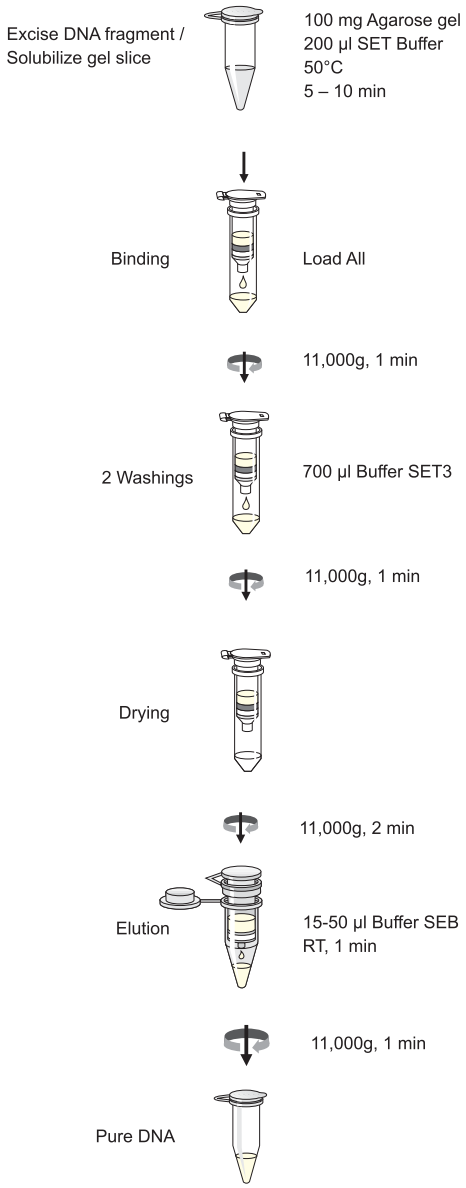
Graph Showing DNA recovery rates of different fragment sizes with different elution volumes

Preparation and Storage of Reagents

The SureExtract® Spin PCR/Gel Extraction Kit kit should be stored at room temperature and is stable for up to one year.

Before starting any SureExtract® Spin PCR/Gel Extraction Kit protocol prepare the following:

Wash Buffer SET3: Add 40ml and 200ml of ethanol (96 – 100 %) to each bottle of Buffer SET3 Concentrate (Kits NP-36105 and NP-36107 respectively). Mark the the bottle(s) as"Ethanol added". Store Wash Buffer SET3 at room temperature (18 – 25 °C) for up to one year.



Protocol for PCR Clean-up

The following protocol is suitable for PCR clean-up as well as concentration and removal of salts, enzymes, etc. from samples without SDS.

Things to do before starting
Check if Wash Buffer SET3 was prepared as per instructions

Procedure

1. **Mix 1 volume of sample with 2 volumes of Buffer SET (e.g., mix 100 µl PCR reaction and 200 µl Buffer SET).** For sample less than 100µl adjust the volume to 100µl using Buffer SET or water. *Note: For removal of DNA fragments > 65 bp, dilutions of Buffer SET can be used (see technical notes).*

2. **Place a SureExtract® Spin PCR/Gel Extraction Kit Column into a Collection Tube (2 ml) and load the sample. Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the collection tube.**

3. **Add 700µl Buffer SET3 to the SureExtract® Spin PCR/Gel Extraction Kit Column. Centrifuge at 11,000 x g for 1 min. Discard flowthrough and place the column back into the collection tube.**

4. **Repeat step 3. Discard flowthrough and place the column back into the collection tube.** Optional: Add 250µl of Buffer Set 3 to the column & centrifuge at 11,000 x g for 2 minutes
Note: A low A_{260}/A_{230} values also indicate carryover of chaotropic salts. Modify wash step.

5. **Centrifuge at 11,000 x g for 2 min for complete removal of Buffer SET3.** Make sure the spin column bottom does not touches the flow-through during handling.
Note: Residual ethanol from Buffer SET3 may inhibit enzymatic reactions. Total removal of ethanol can be achieved by incubating the columns for 2 – 5 min at 70 °C prior to elution.

6. **Place the SureExtract® Spin PCR/Gel Extraction Kit Column into a new 1.5 ml microcentrifuge tube (not provided). Add 15 – 50 µl Buffer SEB (pre warmed at 70°C) allow it to stand at room temperature (18 – 25 °C) for 1 min. Centrifuge for 1 min at 11,000 x g.**
Note: Typical recovery rates for fragment size 50-10Kbp with 15ul of SEB is 70-90% (highly concentrated), for 5-15ug of DNA yield, from more than 100ul of PCR reactions, perform elution with 50ul of preheated SEB.

Protocol for DNA extraction from agarose gels

Things to do before starting
Check if Wash Buffer SET3 was prepared as per instructions.

Procedure

1. **Excise the DNA fragment from an agarose gel using sterile blade/scalpel precisely. For each 100mg of agarose gel add 200 µl Buffer SET.** For gels containing > 2 % agarose, double the volume of Buffer SET. Do not add more than 400mg of gel (or 200mg of >2% gel) per SureExtract® Spin Column is 400 mg or 200 mg of a high percentage gel > 2 %. In this case 2 loading steps are required (step 2). Incubate sample for 5 – 10 min at 50°C, pulse vortexing every 2 – 3 min until the gel slice is completely dissolved.
   Note: Excess UV exposure damages nucleic acids. Weigh the gel slice and transfer it to a clean 1.5ml microcentrifuge tube.

2. **Load sample onto the SureExtract® Spin PCR/Gel Extraction placed in a Collection Tube (2 ml). Centrifuge for 1 min at 11,000 x g. Discard flow-through and place the column back into the collection tube.**

3. **Add 700 µl Buffer SET3 to the SureExtract® Spin PCR/Gel Extraction Column. Centrifuge for 1 min at 11,000 x g. Discard flowthrough and place the column back into the collection tube.**

4. **Repeat step 3. Discard flowthrough and place the column back into the collection tube.**
   Optional: Add 250µl of Buffer Set 3 to the column & centrifuge at 11,000 x g for 2 minutes
   Note: A low A_{260}/A_{230} values also indicate carryover of chaotropic salts. Modify wash step.

5. **Centrifuge for 2 min at 11,000 x g to remove Buffer SET3 completely. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the collection tube.**
   Note: Make sure ethanol (contained in Buffer SET3) is completely removed.Incubating the columns for 2 – 5 min at 70 °C prior to elution helps in complete removal of ethanol.

6. **Add 15-50 µl Buffer SEB to SureExtract® Spin PCR/Gel Extraction Column placed in fresh 1.5 ml microcentrifuge tube (not provided) and allow it to stand for 1 minute at room temperature (18 – 25°C). Centrifuge for 1 min at 11,000 x g.**
   Note: For 5-15ug of DNA yield, from gel slices >200mg, perform elution with 50ul of preheated SEB.

TROUBLESHOOTING GUIDE

Incomplete lysis of agarose slices

Possible cause
- Time and temperature

Suggestion(s):
- Incubation (step 1) can be prolonged up to 20 min according to the size (weight) of gel slice. Pulse vortex every 2 min. Also check incubation temperature.

Low DNA Yield

Possible cause
- Improper reconstitution of reagents

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

Possible cause
- Incompletely dissolved gel slice

Suggestion(s):
- Increase incubation time or add another two volumes of Buffer SET and vortex the tube every 2 minutes during incubation at 50°C.

Possible cause
- Insufficient drying of the silica membrane

Suggestion(s):
- Incubate column for 2 – 5 min at 70°C before elution to remove ethanolic Buffer SET3 completely.

Possible cause
- Low elution buffer SEB

Suggestion(s):
- Especially when larger amounts of DNA (> 5 µg) are bound, increase elution buffer volume up to 100 µl.

Possible cause
- Large DNA fragments

Suggestion(s):
- Preheat Elution Buffer SEB to 70°C, and incubate on the silica membrane at room temperature for 2 min before centrifugation.

TROUBLESHOOTING GUIDE

Appearance of additional bands on agarose gel

Incubate at 95 °C for 2 min and allow the mixture to cool slowly to room temperature (at this step the DNA re-anneals). Add the enzyme and continue with your downstream application. If water is used for elution denaturated (single-stranded) DNA might appear.

Appearance of additional bands on agarose gel

Possible cause

- Carry-over of ethanol/ethanolic Buffer SET3

Suggestion(s):

- Centrifuge 5 min at 11,000 x g or better incubate column for 5 – 10 min at 70 °C before elution to remove ethanolic Buffer SET3 completely

Possible cause:

- Carry-over of chaotropic salts

Suggestions(s):

- Modify washing and / or drying step in case of sensitive downstream applications to remove last traces of Buffer SET

  Add 250 µl Buffer SET3 to the SureExtract® PCR/Gel Extraction Kit Column at the drying step (5).

Low DNA concentration

Possible cause

- DNA was damaged by UV light

Suggestion(s)

- Reduce UV exposure time to a minimum when excising a DNA fragment from an agarose gel.

Low ratio A_{260} / A_{230}

Possible cause

- Carry-over of chaotropic salts

Suggestion(s)

- Refer to detailed troubleshooting "Suboptimal performance of DNA in sequencing, restriction, or ligation reactions - Carry-over of chaotropic salts".

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

SureExtract® Spin PCR/Gel Extraction 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 SureExtract® Spin PCR/Gel Extraction 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 SureExtract® Spin PCR/Gel Extraction 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.

Please contact:
Genetix Biotech Asia (P) Ltd.
71/1, Najafgarh Road, Shivaji Marg,
New Delhi. 110015. INDIA.
e-mail: info@genetixbiotech.com
techsupport@genetixbiotech.com

Tel: +91-11-45027000
Fax: +91-11-25419631

Trademarks:
SureExtract is a registered trademark of Genetix Biotech Asia (P) Ltd.

NOTE:

NOTE:

NOTE: