Instruction Manual
Research Use Only

SPINeasy DNA Purification Kit

For the removal of contaminants inhibiting enzymatic reactions

Size: 50 & 5 preps
Storage: 15-25 °C
Cat. No.: 116548050 (50 PREPS)
116548000 (5 PREPS)
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Table of Contents

1. Introduction to SPINeasy DNA Purification Kit 3
2. Kit Components and User Supplied Materials 4
3. Storage and Kit Stability 5
4. Important Consideration Before Use 5
5. Safety Precautions 6
6. Protocol 7
7. Flow Chart 10
8. Data 11
9. Troubleshooting 12
10. Product Use Limitation & Warranty 14

1. Introduction to SPINeasy DNA Purification Kit

The SPINeasy DNA Purification Kit is designed for fast and efficient cleanup of pre-purified DNA samples contaminated with a wide range inhibitory compound such as those found in environmental sample (humic acid, heavy metals…) as well as chemical compounds which might originate from insufficient purification procedures. Since problematic samples may include diverse amounts of humic acid contaminant, the SPINeasy DNA Purification Kit provides two alternative binding buffers. The Buffer P1 is recommended is designed to purify samples with slight contamination of humic acids (light brownish color) or other PCR inhibitors while the Buffer P1HA include a novel and proprietary humic acid removal technology to process samples considerably contaminated with humic substances (strong brownish color). This kit has been validated with genomic DNA isolated from various problematic soils and with DNA samples spiked with humic acid molecules. However, it performs well on any DNA sample > 200bp. Samples isolated can be readily can be used immediately in downstream applications, including long fragment PCR, qPCR, and next-generation sequencing (16S and whole genome).

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	DNA fragments (>200bp) or genomic DNA contaminated with Inhibitors
Sample amount	up to 25 – 200 µL
DNA recovery	Typically >80 %
Elution volume	30 – 100 µL
Preparation time	< 15 min (6 preps)
Binding Capacity	50 µg

2. Kit Components and User Supplied Materials

2.1 SPINeasy DNA Purification Kit Component

	50 PREPS (Cat.No.: 116548050)		5 PREPS (Cat.No.: 116548000)	
Components	Package	Cat. No.	Package	Cat. No.
Equilibration Buffer	12 mL	116547059	1.2 mL	116547009
Buffer P1	50 mL	116548051	5 mL	116548001
Buffer P1HA	50 mL	116548052	5 mL	116548002
Buffer P2	40 mL	116548053	4 mL	116548003
Buffer P3	40 mL	116548054	4 mL	116548004
Buffer P4	6 mL	116548055	1 mL	116548005
Column P	50 ea	116548056	5 ea	116548006
2.0 mL Collection Tubes	100 ea	116543059	10 ea	116543009
1.5 mL Collection Tubes	50 ea	116543060	5 ea	116543010
Quick-start Protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

2.2 User Supplied Materials

- Microcentrifuge capable of at least 14,000 *g*
- Absolute ethanol (50 mL)
- 1.5 mL microcentrifuge tubes
- UV transilluminator (for gel extraction)
- Razor blade, pen knife or gel cutting device (for gel extraction)
- Heat block or water bath (for gel extraction)
- Isopropanol (optional, for gel extraction)
- Single-channel pipettors (1 μL-1000 μL)
- Nuclease-free, aerosol-preventive tips
- Rack for microcentrifuge tube
- Biohazard disposal containers
- Personal Protective Equipment

3. Storage and Kit Stability

All the components and reagents of the SPINeasy DNA Purification Kit can be stored at room temperature (15-25˚C) until the expiration date printed on the kit label. For extended storage or storage in dry condition (humidity < 40%), store the columns at 2-8˚C to maintain performance.

4. Important Consideration Before Use

- ❑ Use vacuum manifold to accelerate sample processing.

5. Safety Precautions

Wear personal protective equipment (gloves, lab coat and eye protection) to prevent contact with the skin or mucous membranes. Consult the Material Safety Data Sheet at www.mpbio.com for additional details. Buffer P1, P1HA and P2 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. The Buffers P2 and Buffer P3 are flammable.

6. Protocol

Choice of the binding buffer.
-The **Buffer P1** is recommended for samples containing inhibitors from e.g., blood (heme) or plant (polyphenols), etc. DNA including a small amount of inhibitor from soil (humic acid, slight brownish to yellow color) would be also optimally purified.
-The **Buffer P1HA** is only recommended for samples heavily contaminated with humic substances **(**dark brownish color). The use of **Buffer P1HA** in absence of humic acid may decrease the DNA yield by 10-15%.

1. **Column P preparation**

   Add **200 µL Equilibration Buffer** into **Column P** membranes to ensure its performance, centrifuge for **1 min @ 15000 *g***. Discard the flow-through and reuse the collection tube.

2. **Sample preparation**

   A maximum of **50 µg** of DNA resuspended in volume as large as **200 µL** can be used.

   -**Buffer P1**: Mix **5** volumes of **Buffer P1** to **1** volume of the DNA sample in a microcentrifuge tube (not provided).

   -**Buffer P1HA**: **Shake vigorously Buffer P1HA** for **2-5 sec** before use. Mix **5** volumes of **Buffer P1HA** to **1** volume of the DNA sample in a microcentrifuge tube (not provided). **Centrifuge the microcentrifuge tube for 1min @ 15,000 *g* and taking care to avoid the dark pellet,** transfer the entire volume of supernatant to the column P in the next step.

The Buffer P1HA includes a humic acid removal technology that need to be removed along with humic acid contaminants before binding of the DNA to the Column P.

Note: pipet slowly to maximise recovery as the mixture/supernatant including the binding buffer and the DNA sample may be slightly viscous.

The subsequent steps can be performed using either microcentrifuge or vacuum manifold.

Microcentrifuge

3. Binding
Apply the sample to the Column P and centrifuge for **10-30 sec @ 15,000 *g*** and discard the flow-through. Repeat the process if needed until all the lysate has passed through the Column P.

4. 1st Wash
Transfer the Column P into new **2.0 mL collection tubes** (provided). Add **700 µL Buffer P2** to the center of the column, centrifuge for **10-30 sec @ 15,000 *g***. Discard the flow-through and place the Column back into the same 2.0 mL Collection Tube.

5. 2nd Wash
Add **700 µL Buffer P3** to the center of the column and centrifuge for

Vacuum manifold

3. Binding
Insert the Column P into the vacuum manifold's luer connectors. To bind DNA, apply the sample and **apply vacuum**. Repeat until all the lysate has been loaded if required. **Switch off** the vacuum source to avoid membrane overdying.

4. 1st Wash
Add **700 µL Buffer P2** to the center of the column and apply vacuum. **Switch off** the vacuum source.

5. 2nd Wash
Add **700 µL Buffer P3** by running the pipette tip along the wall of the column and **apply vacuum**.

6. Column drying

Transfer Column P into new 2.0 mL Collection Tubes (provided), centrifuge for **2 min @ 15,000 *g***.

7. Elution

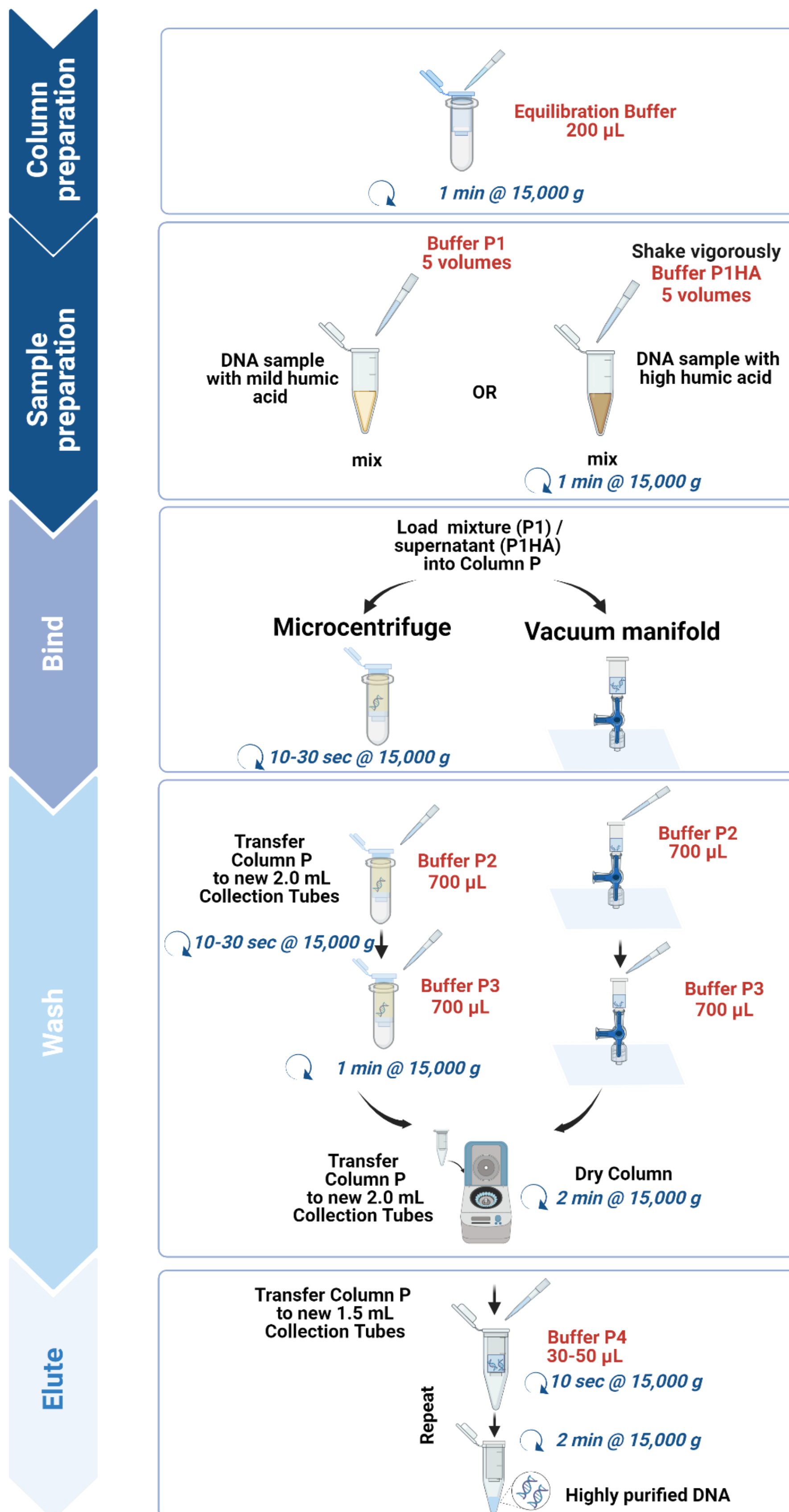
Transfer Column P into new **1.5 mL Collection Tubes** (provided) and add **30-50 µL Buffer P4** to the center of the column, centrifuge for **10 sec @ 15,000 *g***. Recollect the eluate and reload onto the column for a maximal concentration or add fresh **30-50 µL** Buffer P4 for maximal yield. Wait **1min** and spin for **2 min @ 15,000 *g***.

Note: *for genomic DNA or other high molecular weight DNA, it is recommended to wait 5-10 min prior to the second elution in order to maximize the recovery.*

Note: *The elution can be performed using water, but free/thaw cycles may degrade DNA. It is recommended to use the elution buffer provided which is compatible with enzymatic reaction or use 5 mM Tris-HCl buffer PH 8.0.*

Note: The nucleic acid concentration of the sample is calculated from its UV absorbance at 260 nm where an absorbance of 1 (1 cm path length) is equivalent to 50 µL DNA/mL. Contaminations with, RNA, protein, salt, ethanol and humic acids or other non-nucleic acid contaminants contributes to the total absorption at 260 nm and therefore leads to an overestimation of the real DNA concentration. When measured using a UV spectroscopy, a ratio of A260/A280 between 1.80-1.90 and A260/A230 >1.8 indicates pure DNA. A260/A280 and 260/230 ratio above 2.0 indicate RNA contamination. Conversely, an A260/A280 ratio below 1.8 indicates protein contamination. Additionally, a low A260/A230 ratio indicate increasing humic acids, but also proteins, saccharides, ethanol, salt, and other contaminants which may inhibit subsequent enzymatic reaction.

7. Flow Chart



8. Data

An ideal DNA extraction process shall effectively remove inhibitory substances without compromising the DNA yield. Compared to a competitor Z kit, variously contaminated samples purified using the SPINeasy DNA purification kit displayed the highest A260/A280 and A260/A230 purity ratio. Moreover, such samples showed no inhibition in PCR or qPCR, indicating the absence of contaminants and inhibitors.

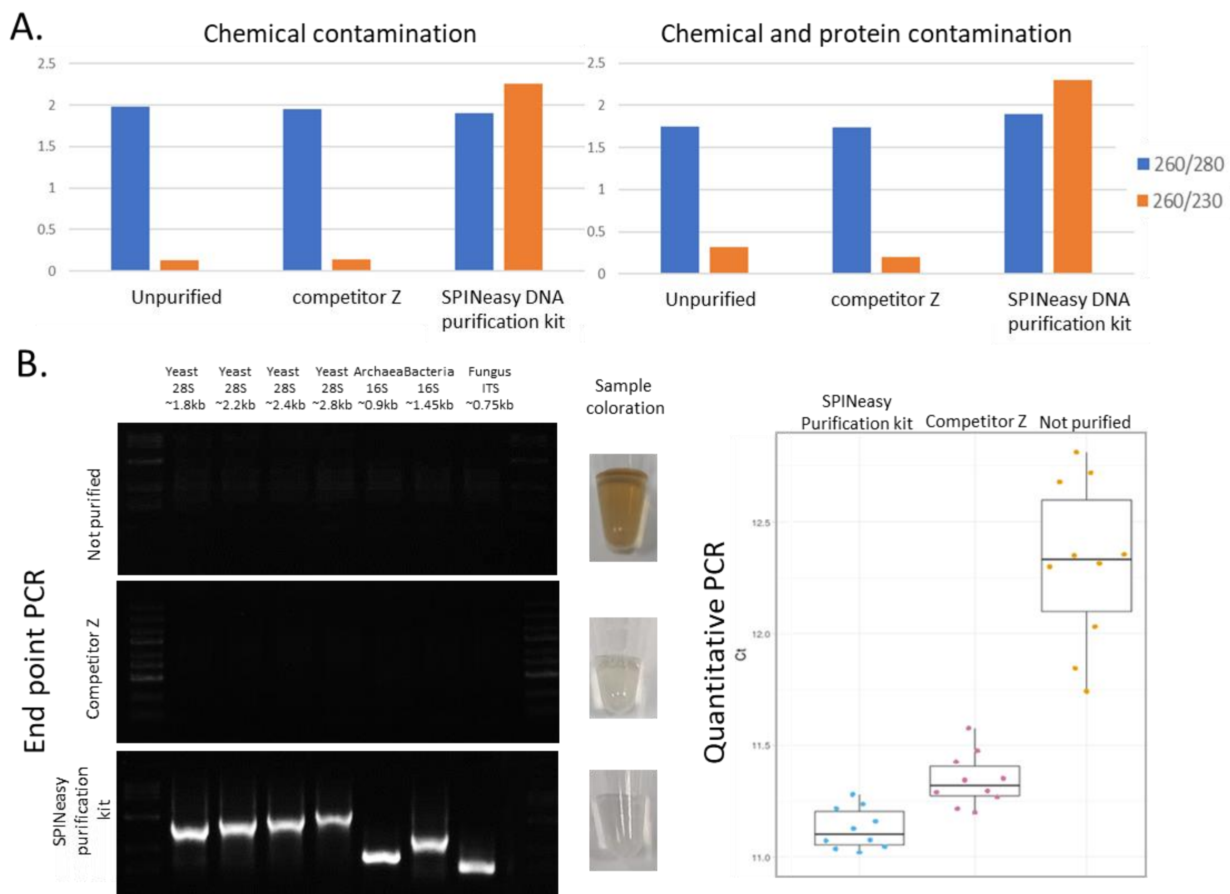


Figure A: SPINeasy DNA Purification Kit improves A260/280 and A260/230 ratios of sample contaminated with chemicals (SDS, chaotropic salts, solvents, humic acid) or both chemicals and proteins.

Figure B: DNA samples spiked with humic acid were purified using SPINeasy DNA Purification Kit or a competitor Z kit. Using end point PCR (left), no amplification was observed in absence of purification but also following competitor Z kit purification due to the presence of inhibitory humic acid. Conversely, all the PCRs were positive using SPINeasy purified DNA, indicating an efficient removal of humic acid. In line with this, a delayed quantitative amplification was observed with competitor Z purified DNA compared to SPINeasy purified DNA (quantitative PCR, right). End point and quantitative PCR were performed in a final volume of 20 µL using ~120 ng and ~50 ng of DNA, respectively.

9. Troubleshooting

This guide may be useful in solving any problems that may arise. For further assistance, please contact our technical support team at **apac-techsupport@mpbio.com**

Problem	Possible Cause	Recommendation
Low DNA Yield	Absence of second elution with the same eluate or fresh buffer	An increase of DNA yield by ~20% can be expected by eluting twice.
	Incorrect quantification of the DNA amount in the provided sample	The presence of inhibitory substances (e.g., humic substances, polyphenols) is known to bias the DNA quantification by spectrophotometry and fluorescent methods leading to a considerable overestimation of DNA, especially in coloured samples or samples with inacceptable A260/280 or A260/230 ratios. Use Agarose gel to estimate the amount of DNA before and after purification.
	Absence of centrifugation after mixing with Buffer P1HA	The humic acid removal technology included in the buffer may decrease the DNA binding to the column P by half if not removed by centrifugation after mixing with Buffer P1HA. Centrifuge after mixing with Buffer P1HA. If the samples do not contain humic acid, the humic acid removal molecule will form a clear pellet that need to be left in the tube and not transferred to the column P.
	Use of Buffer P1HA with sample including no or low humic acid content.	In absence of humic acid, yield may be decreased by ~10% if the Buffer P1HA is used. Use Buffer P1 instead.
Brownish eluate	Inefficient humic acid removal	Use Buffer P1HA instead Buffer P1 for DNA clean up. The Buffer P1HA need to be mixed vigorously immediately before use. If you still observe coloration in your samples despite the use of Buffer P1HA, please contact MP Technical Services.
Low A260/230 or A260/280 ratios	Insufficient amount of DNA used for quantification	To obtain an accurate estimation of the sample purity using spectrophotometer, a concentration of DNA >30-50 ng/µl is required.

Poor PCR Performance	High concentration of DNA	Dilute the DNA sample. Large amount of DNA sample is inhibitory for PCR large amount of DNA molecule in the confined space of the reaction vessel is known to lead to false priming, exhaustion of the magnesium ions, primer(s), dNTP(s) and obstruct the passage of the large Taq polymerase molecules. If PCR using undiluted sample is required, check enzyme specification and manufacturer instruction or choose alternative PCR enzyme with strong strand displacement activity. If PCR can be done using diluted sample, the amount of DNA to be used is specific to each PCR enzyme and may need to be optimized by the user. However, genes in multiple copies in the genome such as ribosomal genes require much lesser DNA input. The SPINeasy DNA purification kit provided positive amplifications from various samples using as much as >200 ng or as little as <0.20 ng of DNA per PCR of 20 µL using inhibitor sensitive PCR enzymes.
	Suboptimal PCR condition.	Verify PCR reagents and protocol with positive control; adjustment on reaction/cycle conditions or primer selection may be necessary following manufacturer recommendation.

10. Product Use Limitation & Warranty

The products presented in this instruction manual are for research or manufacturing use only. They are not to be used as drugs or medical devices to diagnose, cure, mitigate, treat, or prevent diseases in humans or animals, either as part of an accepted course of therapy or in experimental clinical investigation. These products are not to be used as food, food additives or general household items. Purchase of MP Biomedicals products does not grant rights to reproduce, modify, or repackage the products or any derivative thereof to third parties. MP Biomedicals makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery.

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