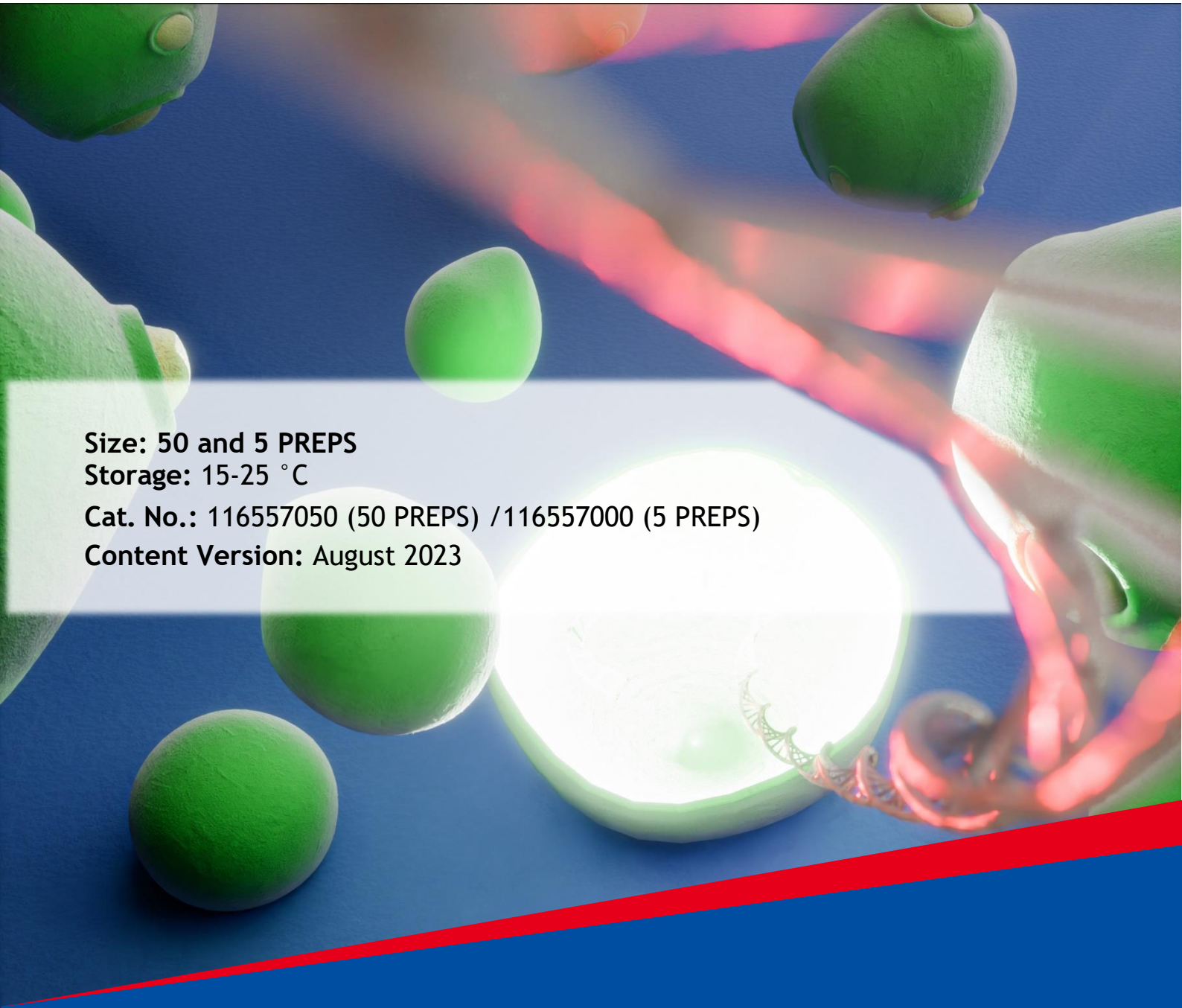
SPINeasy® DNA Kit for Yeast

For the isolation of DNA from Yeast sample



Size: 50 and 5 PREPS
Storage: 15-25 °C
Cat. No.: 116557050 (50 PREPS) /116557000 (5 PREPS)
Content Version: August 2023

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1. Introduction to SPINeasy® DNA Kit for Yeast

SPINeasy® DNA Kit for Yeast is a high-performance genomic DNA (gDNA) extraction kit which is developed based on silica-membrane spin-column technology. This kit enables quick isolation of gDNA from yeast cells, typically in less than 30 minutes. Provided in the kit, Column S and kit buffers are designed to deliver gDNA extracts of high yield and purity; the extracted gDNA is compatible with downstream applications such as PCR amplification, restriction enzyme digestion, and sequencing.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini spin column
Sample	Yeast
Sample amount	up to 4 x 10^8 cells.
Observed yield	up to 12 µg (number of cells and processing dependent)
Elution volume	50–200 µL
Preparation time	30 min

2. Kit Components and User Supplied Materials

2.1 SPINeasy® DNA Kit for Yeast Components

Product	50 reactions (Cat.No.: 116557050)		5 reactions (Cat.No.: 116557000)	
	Package	Cat. No.	Package	Cat. No.
Lysing Matrix YG	50 x 2 mL	116557051	5 x 2 mL	116557001
Lysis Buffer Y	30 mL	116557052	3.0 mL	116557002
Binding Buffer Y	30 mL	116557053	3.0 mL	116557003
Wash Buffer Y1	32 mL	116557054	3.2 mL	116557004
Wash Buffer Y2	4.8 mL	116557055	480 µL	116557005
Elution Buffer EB	12 mL	116552054	1.2 mL	116552004
RNase A	280 µL	116557056	28 µL	116557006
Column S	50 ea	116530058	5 ea	116530008
Collection tube	50 ea	116546059	5 ea	116558009
Elution tube	50 ea	116546060	5 ea	116546010
Quick-Start Protocol	1 each		1 each	
Instruction Manual	Available www.mpbio.com		Available www.mpbio.com	
MSDS & CoA	Available www.mpbio.com		Available www.mpbio.com	

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex
- Microcentrifuge capable of centrifugation of at least 15,000 *g*
- Absolute ethanol
- Single-channel pipettors (5 µL–1000 µL)
- Nuclease-free, aerosol-preventive tips

3. Storage and Kit Stability

The SPINeasy® DNA Kit for Yeast is guaranteed until the expiry date stated on the kit when stored at room temperature (15-25˚C). For extended storage or storage in dry condition (humidity < 40%), store the Column S at 2-8˚C to maintain their performance.

4. Important Consideration Before Use

Please check as appropriate:

☐ Add **40 mL** (50 PREPS kit) or **4 mL** (5 PREPS kit) of absolute ethanol to **Wash Buffer Y2**. Mark on the bottle after addition.

☐ Centrifugation speed stated in the manual will be a guideline; use the maximum speed available if 15,000 *g* is not feasible.

5. Safety Precautions

Lysis Buffer Y, Binding Buffer Y and Wash buffer Y1 contain a component that can be harmful if swallowed and may cause irritation when in contact with skin and eyes. To prevent accidental ingestion, do not eat, drink, or smoke when using this product. 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.

6. Protocol

Things to do before starting.

■ Add indicated amount of absolute ethanol to **Wash Buffer Y2**.

Procedure:

1. Sample Preparation

   a. Harvesting cells from liquid cultures

      Transfer the culture **1.0-2.0 mL** (**maximum 4 x 10^8 cells**) to a microcentrifuge tube. Centrifuge for **5 min** at **15,000 *g*** and discard the supernatant.

   b. Harvesting colonies from solid medium

      Use an inoculating loop to transfer single colony (**≥ 2 mm** in diameter) or colonies (amount **< 50 mg**) from the solid medium.

2. Add **560 µL Lysis Buffer Y** and **5 µL RNase A** to each microcentrifuge tube of pellet. Resuspend pellet with the lysis buffer and RNase A mixture by pipetting repeatedly. Transfer all lysate to **Lysing matrix YG** tube.

3. Homogenize either in a **FastPrep** Instrument for **35 sec** at speed setting of **5.0 m/s** or vortex for **10 min** at speed setting of **2500 rpm**.
   *Note: For cells > **4 x 10^8** or colonies > **50 mg**, homogenize twice.*
4. Centrifuge at **15,000 *g*** for **2 min**.
5. Slowly add **560 µL Binding Buffer Y** to the mixture. Mix thoroughly by inversion.
6. Centrifuge at **15,000 *g*** for **1 min**.

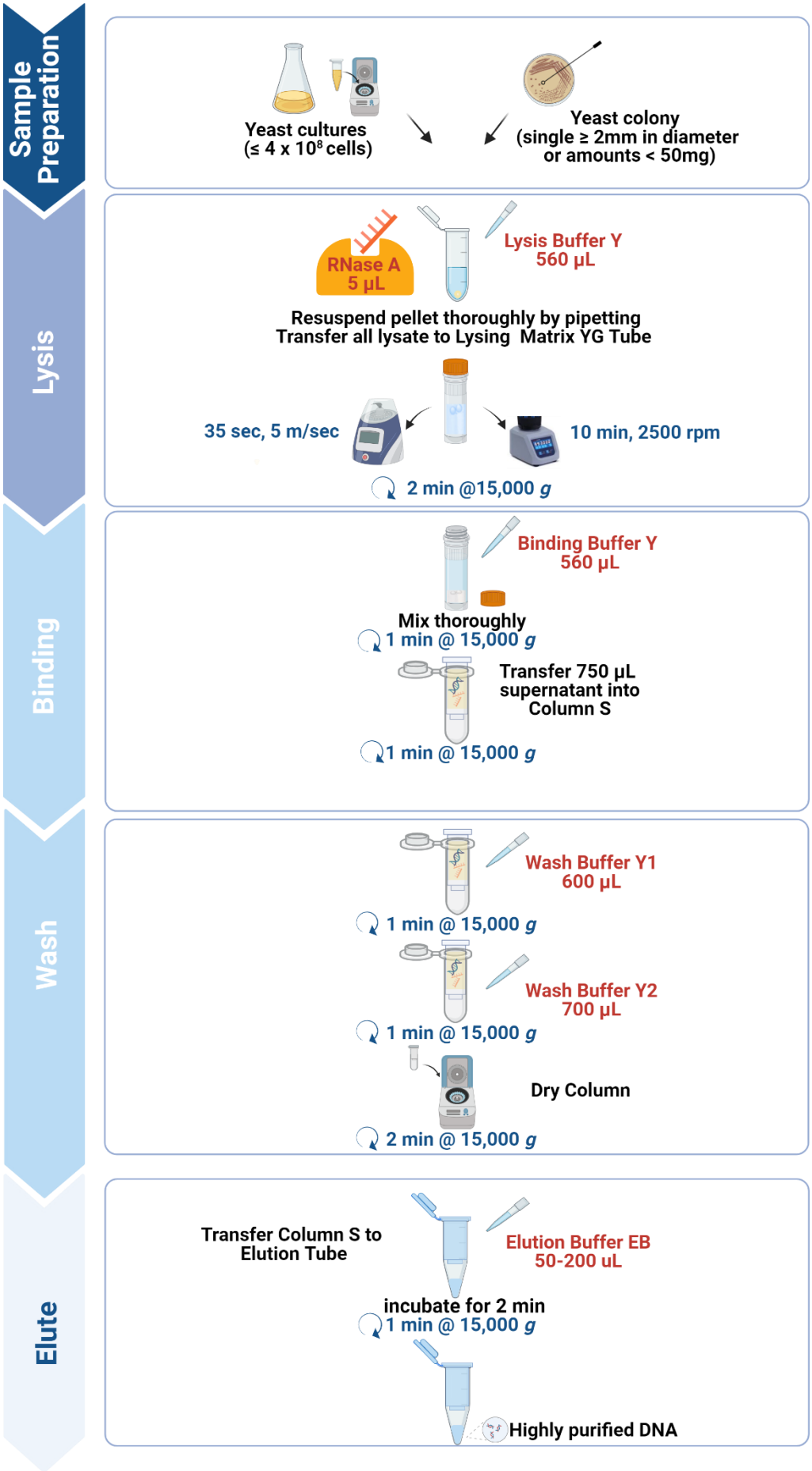
   *Note: To achieve the best performance, add **200 µL 1 M NaOH** (prepared by users) to the*

***Column S** before **step 7**, centrifuge at **15,000 g** for **1 min**. Discard the flow-through.*

7. Carefully transfer **750 µL** of the supernatant to **Column S** placed in a **Collection Tube** (provided). Centrifuge at **15,000 *g*** for **1 min**. Discard the flow-through.
8. Add **600 µL Wash Buffer Y1** to the wall of Column S. Centrifuge at **15,000 *g*** for **1 min**. Discard the flow-through.
9. Add **700 µL Wash Buffer Y2** to the wall of Column S. Centrifuge at **15,000 *g*** for **1 min**. Discard the flow-through.
10. Centrifuge at **15,000 *g*** for another **2 min** to dry the column.
11. Transfer Column S into a clean **1.5 mL Elution tube** (provided). Add **50–200 µL Elution Buffer EB** or ultrapure water (**pH > 7.0**) (not provided) to the center of Column S, incubate for **2 min**, and centrifuge at **15,000 *g*** for **1 min** to collect the eluted DNA.

    ***Optional:** For maximum DNA recovery, reload the eluted DNA back to the center of Column S and centrifuge at **15,000 g** for **1 min** to collect the eluted DNA.*

7. Flow Chart

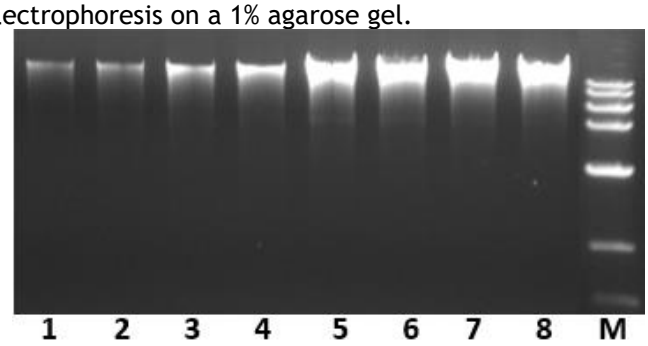

Sample Preparation
Yeast cultures
(≤ 4 x 10⁸ cells)
Yeast colony
(single ≥ 2mm in diameter
or amounts < 50mg)
Lysis
RNase A
5 µL
Lysis Buffer Y
560 µL
Resuspend pellet thoroughly by pipetting
Transfer all lysate to Lysing Matrix YG Tube
35 sec, 5 m/sec
10 min, 2500 rpm
2 min @15,000 g
Binding
Binding Buffer Y
560 µL
Mix thoroughly
1 min @ 15,000 g
Transfer 750 µL
supernatant into
Column S
1 min @ 15,000 g
Wash
Wash Buffer Y1
600 µL
1 min @ 15,000 g
Wash Buffer Y2
700 µL
1 min @ 15,000 g
Dry Column
2 min @ 15,000 g
Elute
Transfer Column S to
Elution Tube
Elution Buffer EB
50-200 uL
incubate for 2 min
1 min @ 15,000 g
Highly purified DNA

8. Data

SPINeasy® DNA Kit for Yeast delivers DNA yield with excellent purity that is in proportion with the input sample amount of *Saccharomyces cerevisiae* (1 x 10^7 to 4 x 10^8 cells). The 260/280 and 260/230 ratios obtained from the extracted DNA are within the acceptable range (Figure 1).

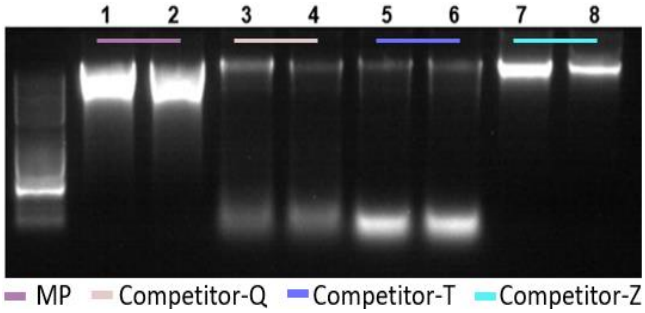
SPINeasy® DNA Kit for Yeast delivers DNA yield doubled that obtained from Competitor Z. Despite Competitor Q and T giving higher spectrophotometric DNA yield, agarose gel electrophoresis result shows that the DNA samples are degraded. SPINeasy® DNA for Yeast gave higher purity ratios of 260/280 and 260/230 than all the competitor's kits (Figure 2).

Figure 1: SPINeasy® DNA Kit for Yeast generates DNA yield that is proportionate to the input sample amount, *Saccharomyces cerevisiae* (1 x 10^7 to 4 x 10^8 cells). 3 µL of purified genomic DNA from a 100 µL eluate were analyzed by electrophoresis on a 1% agarose gel.



Sample	No.	Concentration (ng/µL)	A260/A280	A260/A230
1-2 x 10^7 cells	1	12.784	1.87	2.16
	2	15.396	1.86	2.08
2-5 x 10^7 cells	3	29.149	1.89	2.20
	4	27.321	1.87	2.17
1-2 x 10^8 cells	5	66.683	1.88	2.27
	6	66.645	1.89	2.25
2-4 x 10^8 cells	7	115.567	1.88	2.15
	8	125.671	1.90	2.06

Figure 2: Genomic DNA extracted from *Saccharomyces cerevisiae* (5x10^7 cells). The SPINeasy® DNA Kit for Yeast was used to extract genomic DNA (gDNA) from Saccharomyces cerevisiae. The yield and purity were assessed and compared with three different competitor kits (Q, T, Z). The MP Kit exhibited a purity range exceeding 1.8 while maintaining a high yield. Competitors Q and T yielded high quantities of gDNA but with a lower A260/A230 ratio. Competitor Z yielded both a lower quantity of gDNA and a lower purity ratio. Gel electrophoresis of the extracted gDNA displays maximum band integrity for the MP Kit.



Sample	No.	Concentration (ng/µL)	A260/A280	A260/A230
MP	1	60.363	1.87	1.91
	2	61.138	1.87	2.05
Competitor Q	3	72.729	2.05	0.62
	4	74.359	2.10	0.61
Competitor T	5	100.989	2.12	1.39
	6	95.493	2.14	1.61
Competitor Z	7	32.146	1.85	1.34
	8	22.194	1.75	1.02

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA Yield	Incomplete sample lysis or homogenization	Reduce the number of samples or increase the lysis time.
	DNA inefficiently eluted	Perform elution twice, 100 µL elution buffer each time, for a total elution volume of 200 µL.
	Water of incorrect pH used for elution	The low pH of deionized water from some water purifiers may reduce DNA yield. If eluting with water, ensure that the pH of the water is at least 7.0.
	DNA not efficiently bound to column	Before use, equilibrate the column with 200 µL of 1 M NaOH, and centrifuge 1 minute at the maximum speed. Discard the flow-through.
Smeared DNA band	Yeast sample is too old	Use freshly collected yeast culture. Avoid repeated freeze/thaw cycles of the sample.
	Mechanical sample disruption is too vigorous	If extracting from yeast cells < $1x10^7$, alternative lysis method can be used: lysis by vortexing at the speed of 2000 rpm for 5 minutes.
Low A260/A230 ratios	Carry over ethanol in eluant	Lengthen the column-drying time or increase the centrifugation speed to ensure complete drying of the column membrane and transfer the column into the elution tube carefully so that the column does not come into contact with the flow-through.
	Incomplete purification (inadequate lysis / overloading of column)	Reduce the amount of starting material
High A260/A230 ratios	EDTA in eluant	EDTA present in Elution Buffer EB may be associated with higher than usual A260/A230 ratio and this will not have any negative effect on downstream applications.
Poor PCR Performance	Too much DNA used	Check and adjust the DNA concentration.
	High inhibitor	Dilute template DNA to reduce the inhibitors.
	PCR protocol or reagents were not optimal	Verify PCR reagents and protocol with positive control; adjustment on reaction conditions or primer selection may be necessary.

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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