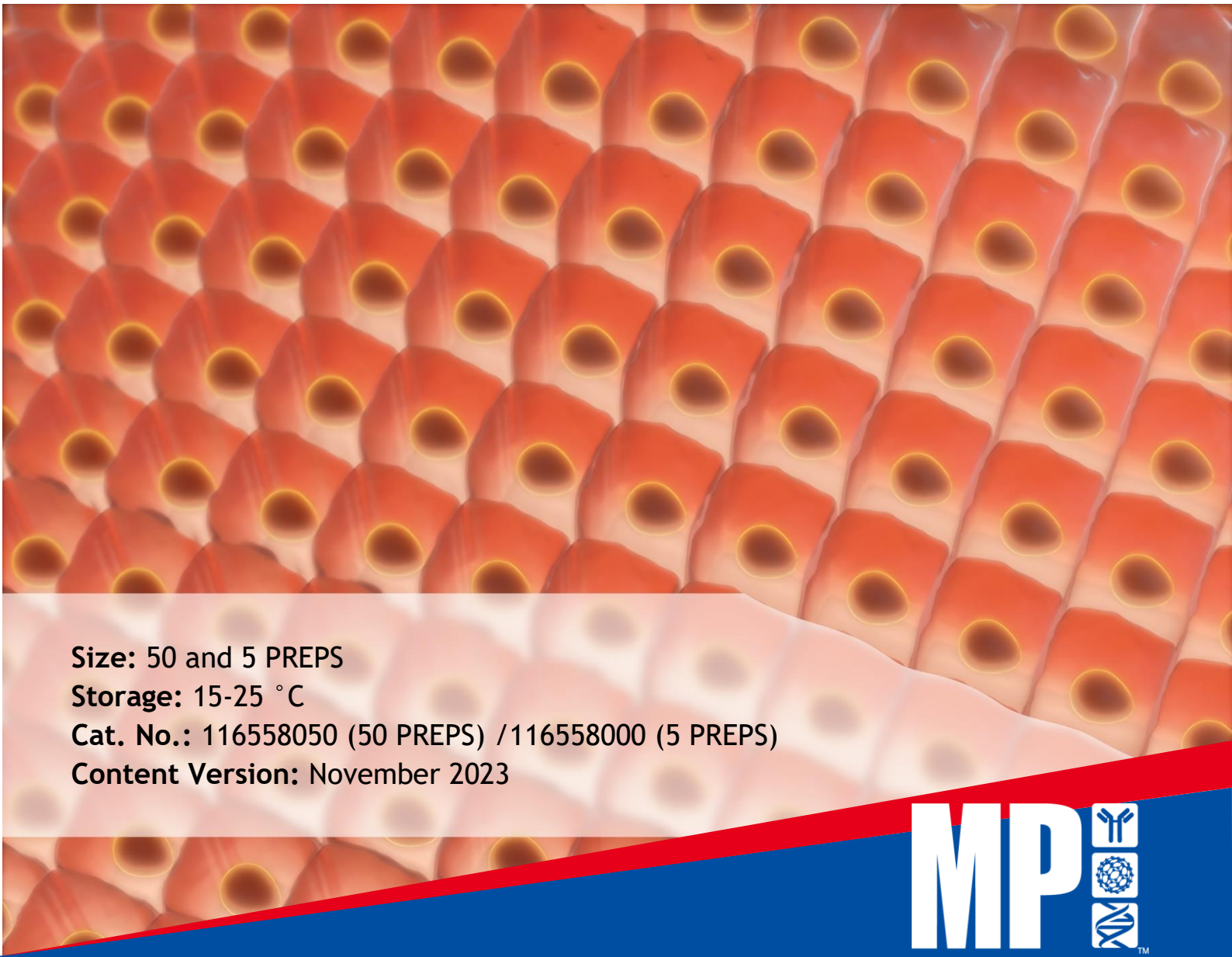
Instruction Manual
Research Use Only

SPINeasy® DNA Kit for Tissue (With Lysing Matrix)

For Simple and Fast Isolation of Genomic DNA from Tissue Samples in 30 Minutes



Size: 50 and 5 PREPS
Storage: 15-25 °C
Cat. No.: 116558050 (50 PREPS) /116558000 (5 PREPS)
Content Version: November 2023

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1. Introduction to SPINeasy® DNA Kit for Tissue (With Lysing Matrix)

SPINeasy® DNA Kit for Tissue (With Lysing Matrix) efficiently isolates high-quality genomic DNA from various types of tissue in 30 minutes. Samples are rapidly disrupted by bead beating method along with Lysing Matrix M and Buffer TD1. It is highly recommended to use FastPrep® Instrument from MP Biomedicals to disrupt the tissue for an optimal yield. The lysis buffer mix containing Proteinase K can completely dissolve the sample in a short 10-min incubation. The combination of mechanical, chemical, and enzymatic lysing methods of this kit provides significantly higher yields of genomic DNA as compared to other commercial kits that have no mechanical lysis. With this optimized lysis method, the hard-to-lyse samples such as tough (rodent tail, ear punches), and fibrous (heart, muscle) tissues can now be easily and completely lysed. The lysis buffer mix also contains RNase A, which minimizes the possibility of RNA contamination. Subsequent treatment with Buffer TD2 enables selective binding of DNA to Column S. The gDNA extracted from multiple types of tissues showed no inhibition in PCR and is immediately ready for downstream applications, including long fragment PCR, qPCR, restriction digestion and sequencing.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini Column
Sample	Animal Tissue (e.g., liver, kidney, muscle, tail)
Sample amount	up to 30 mg
Observed yield	up to 70 µg DNA (sample-dependent)
Elution volume	50–100 µL
Preparation time	30 min

2. Kit Components and User Supplied Materials

2.1 SPINeasy® DNA Kit for Tissue (With Lysing Matrix) Component

Component	50 PREPS (Cat.No.: 116558050)		5 PREPS (Cat.No.: 116558000)	
	Package	Cat. No.	Package	Cat. No.
Lysing Matrix M	50 ea	116923050	5 ea	116923005
Equilibration Buffer	12 mL	116547059	1.2 mL	116547009
Buffer TD1	15 mL	116558051	1.5 mL	116558001
Buffer TD2	30 mL	116558052	3 mL	116558002
Buffer TD3	18 mL	116558053	1.8 mL	116558003
Buffer TD4	6 mL	116558054	600 µL	116558004
Buffer TD5	15 mL	116558055	1.5 mL	116558005
Proteinase K	1.2 mL	116558056	120 µL	116558006
RNase A	250 µL	116558057	25 µL	116558007
Column S	50 ea	116530058	5 ea	116530008
Collection tube	150 ea	116546059	15 ea	116558009
Elution tube	50 ea	116546060	5 ea	116546010
Quick-Start Protocol	1 ea	-	1 ea	-
Instruction Manual	Available www.mpbio.com			
MSDS & CoA	Available www.mpbio.com			

2.2 User Supplied Materials

- FastPrep® Instrument - FastPrep-24™ 5G (Cat. No.116005500) or Vortex with a maximum speed ≥ 2,500 rpm
- ThermoMixer
- Microcentrifuge capable of spinning at ≥ 14,000 *g*
- Absolute ethanol (62 mL)
- Single-channel pipettors (2 µL–1000 µL) and Nuclease-free certified filter tips
- Optional: a commercial vacuum manifold with luer connectors connected to a vacuum pump

3. Storage and Kit Stability

Store Proteinase K at 2-8˚C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15-25˚C) does not affect their performance. The remaining kit components are 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

- ❑ Add 12 mL (1.2 mL for sample kit) absolute ethanol into Buffer TD3 and mark the bottle.
- ❑ Add 50 mL (5 mL for sample kit) absolute ethanol into Buffer TD4 and mark the bottle.
- ❑ The SPINeasy® DNA Kit for Tissue (With Lysing Matrix) requires the use of a centrifuge capable of generating at least 14,000 *g* to obtain optimal results. Use the maximum speed available if 14,000 *g* is not feasible.
- ❑ If FastPrep-24™ 5G (Cat. No.116005500) is not available, the use of a vortex capable of achieving 2,500 rpm is required.

5. Safety Precaution

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. The Equilibration Buffer is corrosive and may cause skin burns and eye damage. Buffers TD1, TD2, TD3 and TD4 can be harmful if swallowed and may cause irritation when in contact with skin and eyes. Buffers TD1, TD2 and TD3 include chaotropic salts, which can form highly reactive compounds when combined with bleach. After adding pure ethanol, Buffers TD3 and TD4 are flammable.

6. Protocol

1. Column preparation

Optional: ***Column S*** *can be pre-treated prior to usage to ensure optimal performance. For this, transfer the Column S into a* ***Collection tube*** *(provided). Add* ***200 µL Equilibration Buffer*** *to the Column S membrane.*

Wait for at least **1 min** and centrifuge for **30 sec @ 14,000 *g***. Transfer Column S into a new **Collection tube**. The treated Column S can be stored at 2-8 ˚C for up to 7 days, if required.

Note: *Column preparation is recommended when higher DNA yield is desired or when column performance is reduced after long-term storage.*

2. Sample preparation

Weigh the appropriate tissue amount (up to **10 mg** for spleen tissue, up to **30 mg** for other tissues) and add them into a **Lysing Matrix M** tube.

Add **200 µL Buffer TD1**, **20 µL Proteinase K** and **4 µL RNase A**. Vortex for **5 sec** to mix well. Briefly spin down the mixture.

Note: *It is not necessary to cut the tissue into small pieces unless the tissue is particularly tough.*

3. Homogenization

Homogenize using FastPrep® for **5 sec, 4 m/sec** or vortex for **5 min @ 2,500 rpm**. Briefly spin down the lysate.

Incubate in a **Thermomixer** at **1,000 rpm** for **10 min at 56°C**.

Optional: *If there is still residual tissue left, repeat the homogenization process one more time with the same settings.*

Note: *The performance of the DNA output (yield, purity, and DNA integrity) obtained using vortex is highly dependent on the model of vortex used. The time and speed can be further optimized by the user. It is recommended to perform vortexing with the use of an adapter (to hold the vials).*

4. DNA binding

Add **500 µL Buffer TD2** into the **Lysing Matrix M** tube with lysate. Mix thoroughly by pipetting up and down for **10 times** or vortex for **10 sec**. Briefly spin down the mixture.

Spin method:

Load all the mixture (**~700 µL**) onto the center of Column S (assembled with Collection tube). Centrifuge for **30 sec @ 14,000 *g***. Discard flow-through and place Column S back into the same Collection tube.

Vacuum method:

Insert Column S into the connector on the vacuum manifold. Carefully add the mixture (**~700 µL**) onto the center of Column S. Switch on the vacuum pump. After the lysate passes through the column completely, switch off the vacuum pump.

5. Wash.

Spin method:

Add **500 µL Buffer TD3** onto the center of Column S, centrifuge for **30 sec @ 14,000 *g***. Discard the flow through and place Column S back into the same Collection tubes.

Add **500 µL Buffer TD4** onto the center of Column S, centrifuge for **30 sec @ 14,000 *g***. Discard the flow through and place Column S back into the same Collection tube. (**Repeat this step once**)

Vacuum method:

Add **500 µL Buffer TD3** onto the center of Column S. Switch on the vacuum pump. After **Buffer TD3** passes through the column completely, switch off the vacuum pump.

Add **500 µL Buffer TD4** onto the center of Column S. Switch on the vacuum pump. After **Buffer TD4** passes through the column completely, switch off the vacuum pump. **(Repeat this step once)**

6. Drying.

Transfer Column S to a new Collection tube and spin for **2 min @ maximum speed.**

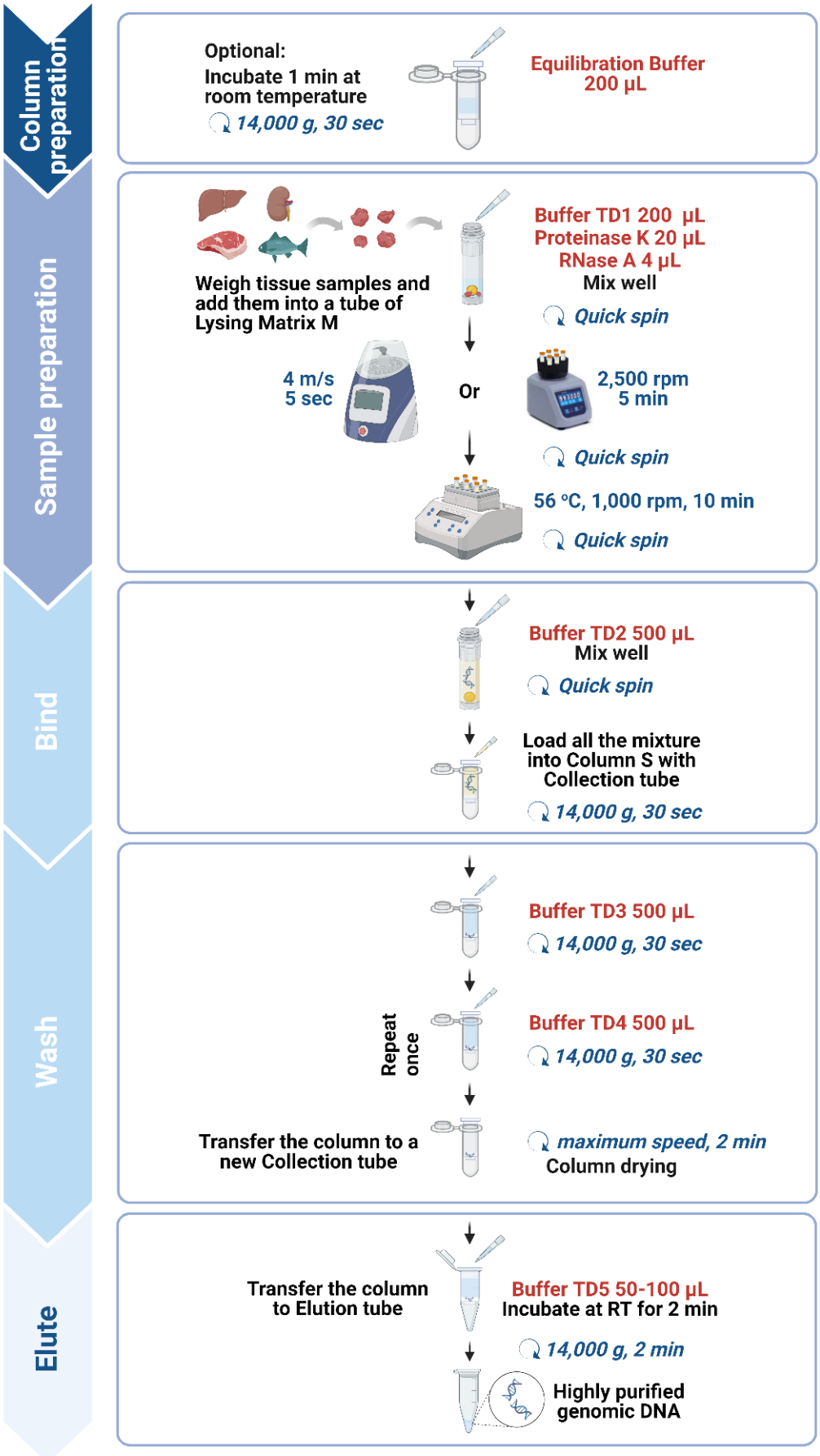
7. Elution

Transfer Column S to **Elution tube**. Add **100 µL Buffer TD5** onto the center of Column S, wait for **2 min** and centrifuge for **2 min @ 14,000 *g*.** Purified DNA is now ready for downstream applications.

Optional: *perform a second elution step with a further **100 µL Buffer TD5** will increase yields by up to 20%).*

Note: *For samples with low DNA content, reduce the elution volume to 50 µL in order to achieve increased concentration of eluted DNA.*

7. Flow Chart


Column preparation
Optional:
Incubate 1 min at room temperature
14,000 g, 30 sec
Equilibration Buffer 200 µL
Sample preparation
Weigh tissue samples and add them into a tube of Lysing Matrix M
Buffer TD1 200 µL
Proteinase K 20 µL
RNase A 4 µL
Mix well
Quick spin
4 m/s 5 sec
Or
2,500 rpm 5 min
Quick spin
56 °C, 1,000 rpm, 10 min
Quick spin
Bind
Buffer TD2 500 µL
Mix well
Quick spin
Load all the mixture into Column S with Collection tube
14,000 g, 30 sec
Wash
Buffer TD3 500 µL
14,000 g, 30 sec
Repeat once
Buffer TD4 500 µL
14,000 g, 30 sec
Transfer the column to a new Collection tube
maximum speed, 2 min
Column drying
Elute
Transfer the column to Elution tube
Buffer TD5 50-100 µL
Incubate at RT for 2 min
14,000 g, 2 min
Highly purified genomic DNA

8. Data

The SPINeasy® DNA Kit for Tissue (With Lysing Matrix) has been rigorously tested for its performance. DNA was extracted from soft (liver), fibrous (heart), tough (mouse tail and fish gill), and fatty-rich tissue (beef) with SPINeasy® DNA Kit for Tissue (With Lysing Matrix) and competitor Q kit. The SPINeasy® DNA Kit for Tissue (With Lysing Matrix) provided high DNA yields along with optimal A260/A280 and A260/A230 ratio, indicating a high extraction performance across a wide range of samples (Figure 1).

The SPINeasy® DNA Kit for Tissue (With Lysing Matrix) consistently yielded higher results when compared to Competitor Q kit across all sample types (Figure 1). It also demonstrated better DNA purity as the A260/A230 ratio was closer to 2.0 as compared to the competitor Q kit. The DNA integrity (DIN) values were equivalent for samples extracted with both kits.

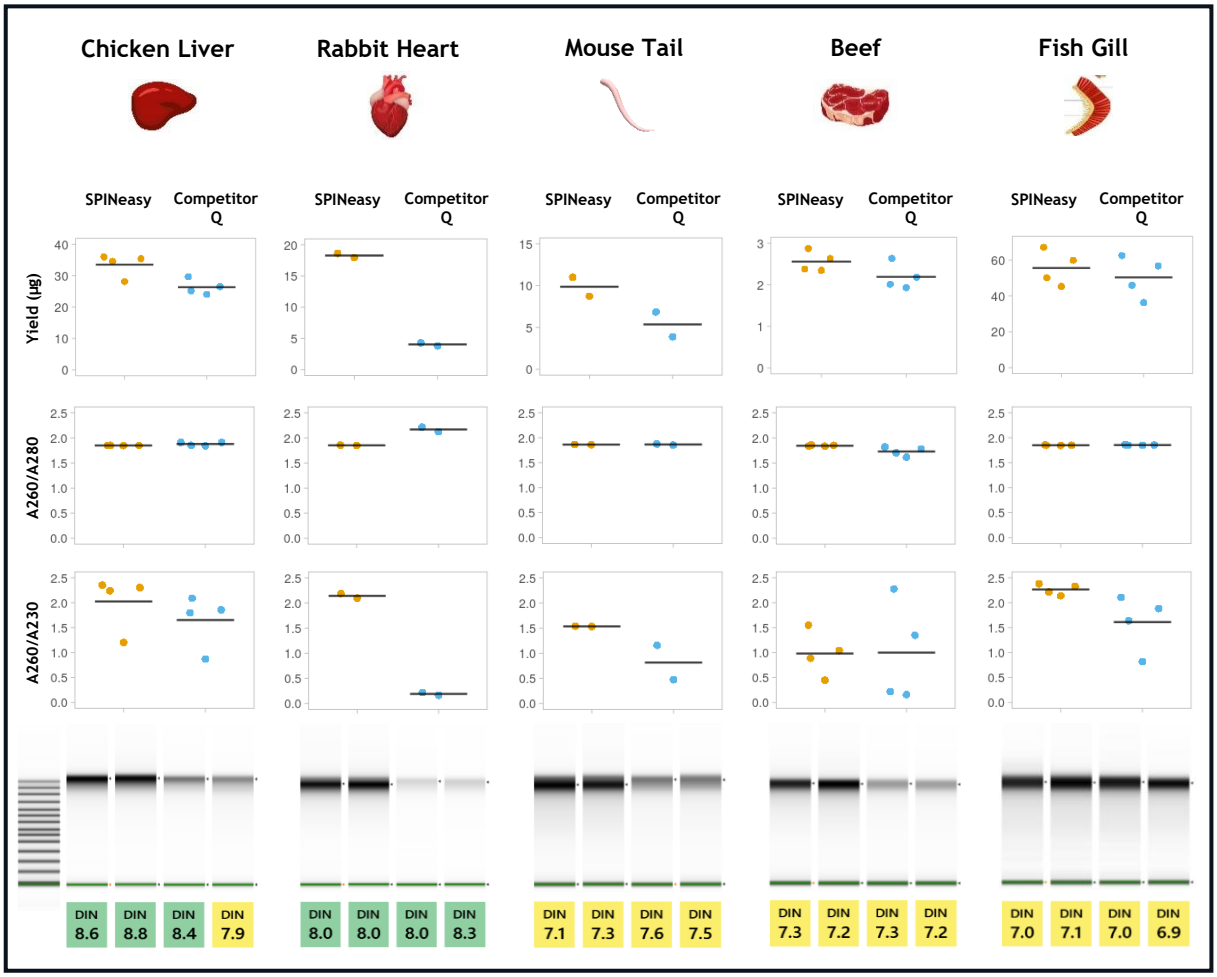


Figure 1: Comparison of DNA quality and quantity of gDNA extracted from various types of tissues using SPINeasy® DNA Kit for Tissue (With Lysing Matrix) and competitor Q kit. The gDNA yield, A260/A230 and A260/A280 ratio (from 15 mg of corresponding tissue types) were measured using a spectrometer. The virtual gel image and DNA integrity Number (DIN, in green and yellow) were analyzed using Agilent

tapestation 4150.

DNA isolated from various rabbit and mouse tissues using the SPINeasy® DNA Kit for Tissue (With Lysing Matrix) can be readily used in long fragment PCR without any inhibition observed (Figure 2, upper panel).

The gDNA obtained from this kit is also compatible for real-time qPCR (Figure 2, lower panel). The Ct values of gDNA isolated from three different tissues (extracted using the SPINeasy® DNA Kit for Tissue (With Lysing Matrix) and the Competitor Q kit) were similar, indicating comparable amplifiability.

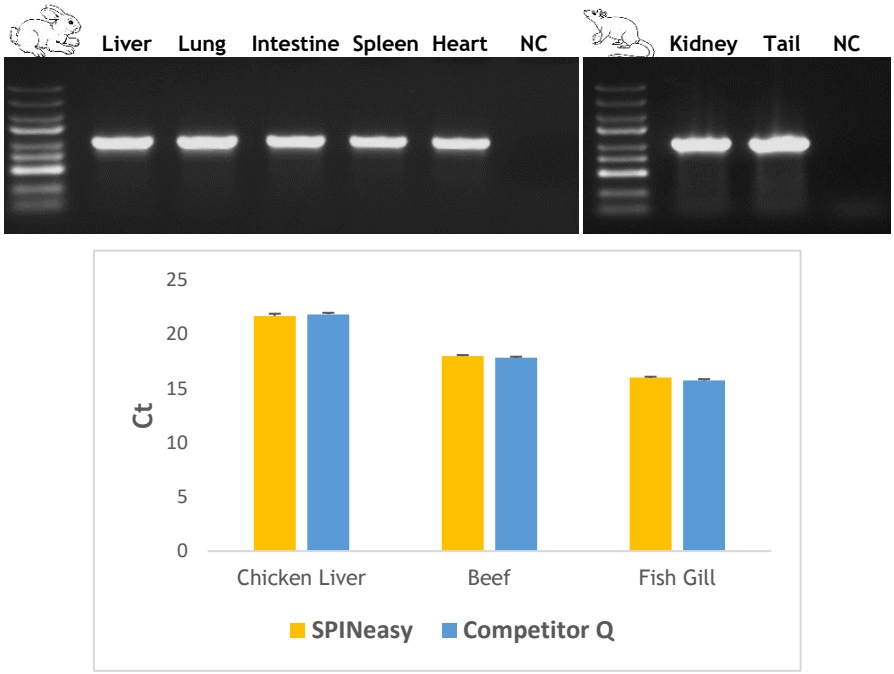


Figure 2: Amplifiability of DNA extracted with SPINeasy® DNA Kit for Tissue (With Lysing Matrix)
Upper panel: Agarose gel electrophoresis of gDNA isolated from various rabbit/mouse tissues using SPINeasy® DNA Kit for Tissue (With Lysing Matrix). One microliter of extracted gDNA was amplified using rabbit or mouse-specific α-actin primers (1kb product). Reactions without DNA served as negative controls (NC).
Lower panel: Comparison of threshold cycles (Ct) of qPCR when amplification was performed using equal quantity of chicken liver, beef and fish gill gDNA (25 ng). Targets were amplified with SYBR green.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA Yield / reduced integrity	Insufficient cell lysis	While a FastPrep® speed setting of 4.0 m/s for 5 seconds is sufficient for most sample types, some samples may require harsher conditions for complete lysis. Homogenization speed and/or time can be increased for such samples. Lysis duration can also be extended when samples are lysed by vortexing.
	Poor elution	Wait for 10 min after addition of Buffer TD5 before centrifuging. Ensure that Buffer TD5 is added to the center of the column membrane.
	Ethanol carry-over	Incubate column at 55°C for 3-5 mins to dry the membrane completely before elution.
	Sample degradation	Fresh or freshly frozen sample is preferred to obtain optimal yield and integrity. It is recommended to store samples frozen in aliquots and avoid repeated freeze-thawing.
	Tissue has low DNA content	Increase the amount of starting material. Process multiple samples using several Lysing Matrix tubes and then pool the samples. Elute in a smaller volume (50 µL).
	Prolonged storage/suboptimal storage condition of Column TD	Pre-treat the column with Equilibration Buffer (refer to 6.1).
Low A260/A280 or A260/A230 ratios	Inaccurate readings due to low DNA concentration	The readings of A260/A230 or A260/A280 may be inaccurate when low concentration < 40 ng/µl of DNA is being measured using a spectrophotometer.
	Clogged Column	Decrease the amount of starting material.
	Insufficient cell lysis	While a FastPrep® speed setting of 4.0 m/s for 5 seconds is sufficient for most sample types, some samples may require harsher conditions for complete lysis. Homogenization speed and/or time can be increased for such samples. Lysis duration can also be extended when samples are lysed by vortexing.

	Contaminants not removed efficiently	After adding Buffer TD4, incubate the Column S at room temperature for 1 min before centrifuging. Ensure that all traces of wash buffer are removed from rim of the Column S prior to elution.To prevent Ethanol carry-over, incubate column at 55°C for 3-5 mins to dry the membrane completely before elution.
High A260/A280	RNA contamination	If the correct amount of RNase A was added, the sample may be a high-RNA content tissue such that the RNase digestion is incomplete. Increasing the RNase amount in the lysis is recommended.
Sheared DNA	Sample over-lysis	Using a vortex instead of a FastPrep® will generally result in a higher DNA integrity but possibly compromised yields.
	Sample degradation	Fresh or freshly frozen sample is preferred to obtain optimal yield and integrity. It is recommended to store samples frozen in aliquots and avoid repeated freeze-thawing.
Poor PCR Performance	High concentration of nucleic acid	Dilute the sample. Large amount of nucleic acid sample is inhibitory for PCR. If PCR using undiluted sample is required, check enzyme specification and manufacturer instruction or choose alternative PCR enzyme with strong strand displacement activity.
	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 in order 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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