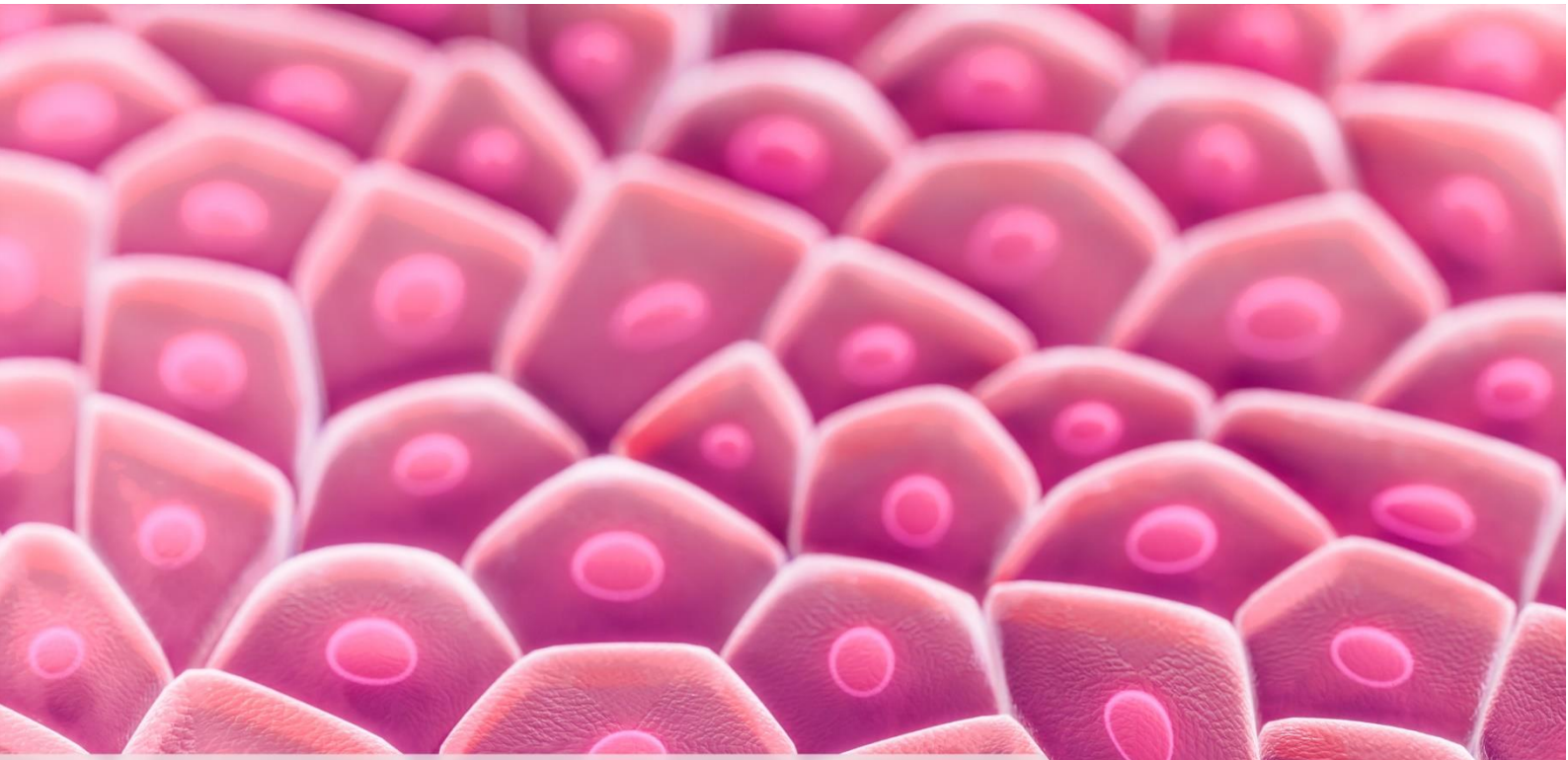
Instruction Manual
Research Use Only

SPINeasy® DNA Kit for Tissue (Without Lysing Matrix)

For Convenient Isolation of Genomic DNA from Tissue Samples



Size: 50 PREPS and 5 PREPS
Storage: 15-25 °C
Cat. No.: 116559050 (50 PREPS) /116559000 (5 PREPS)
Content Version: November 2023

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1. Introduction to SPINeasy® DNA Kit for Tissue (Without Lysing Matrix)

The SPINeasy® DNA Kit for Tissue (Without Lysing Matrix) is a high-performance genomic DNA (gDNA) extraction kit which is based on silica-membrane spin-column technology. This kit enables convenient isolation of gDNA from a variety of solid tissues. The DNA purification protocol does not require any sophisticated equipment. Our specially formulated Buffer TD1 and Proteinase K provide effective lysis of various types of samples including tough, fibrous, and fatty tissues. The RNase A treatment (after lysis step) ensures the removal of potential RNA contamination. Furthermore, the subsequent addition of Buffer TD2 enables selective binding of DNA to Column S. The gDNA extracted from multiple types of tissues using this kit showed high integrity and purity which can immediately be used for downstream applications, including long fragment PCR, real time PCR, restriction enzyme digestion and sequencing.

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Kit Specifications at a Glance

Technology	Silica membrane technology
Format	Mini Column
Sample	Solid 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	1-4 hours

2. Kit Components and User Supplied Materials

2.1 SPINeasy® DNA Kit for Tissue (Without Lysing Matrix) Component

Component	50 PREPS (Cat.No.: 116559050)		5 PREPS (Cat.No.: 116559000)	
	Package	Cat. No.	Package	Cat. No.
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

- Heat block
- Vortex
- 1.5 mL centrifuge tubes
- 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 stable 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 Columns S at 2-8˚C to maintain their performance.

4. Important Considerations Before Use

- ❑ Add 12 mL (1.2 mL for sample kit) of absolute ethanol into Buffer TD3 and mark the bottle.
- ❑ Add 50 mL (5 mL for sample kit) of absolute ethanol into Buffer TD4 and mark the bottle.
- ❑ The SPINeasy® DNA Kit for Tissue (Without 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.

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 burn 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 contain chaotropic salts, which can form highly reactive compounds when combined with bleach. After addition of 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 their optimal performance. For this, transfer Column S into a* ***Collection tube*** *(provided). Add* ***200 µL Equilibration Buffer*** *onto 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 Columns 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 and cut the tissue (up to 10 mg for spleen tissue, up to 30 mg for other tissues) into small pieces and add them into a clean **1.5 mL centrifuge tube** (not-provided).

Add **200 µL Buffer TD1** and **20 µL Proteinase K** into the tissue sample tube. Vortex for **5 sec** to mix well. Briefly spin down the mixture.

Note: The typical recommended starting amount is ***5-10 mg*** *for spleen,* ***15-20 mg*** *for other tissues. It is recommended to cut the tissue into small pieces to increase the lysis efficiency.*

3. Lysate preparation

Incubate the tube in a **Heat block** at **56ºC** for **1 to 3 hrs** or **until the tissue is completely dissolved**. Briefly spin down the lysate.

During the incubation, vortex intermittently facilitates tissue digestion.

Note: If the tissue is hard to digest, increasing the incubation temperature to 65˚C may accelerate the lysis process.

Add **4 µL RNase A** and vortex for **5 sec** to mix well. Incubate at **room temperature** for **2 min**. Briefly spin down the lysate.

4. DNA binding

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

Centrifuge:

Assemble Column S into Collection tube.

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

Vacuum manifold:

Insert Column S into the connector on the vacuum manifold. Carefully load all the mixture (**~700 µL**) onto the center of the column.

Switch on the vacuum pump. After all the lysate passes through the column, switch off the vacuum pump.

5. Wash.

Centrifuge:

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 tube.

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

Vacuum manifold:

Add **500 µL Buffer TD3** onto the center of Column S. Switch on the vacuum pump. After **Buffer TD3** has passed 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** has passed through the column completely, switch off the vacuum pump. **(Repeat this step once)**

6. Drying.

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

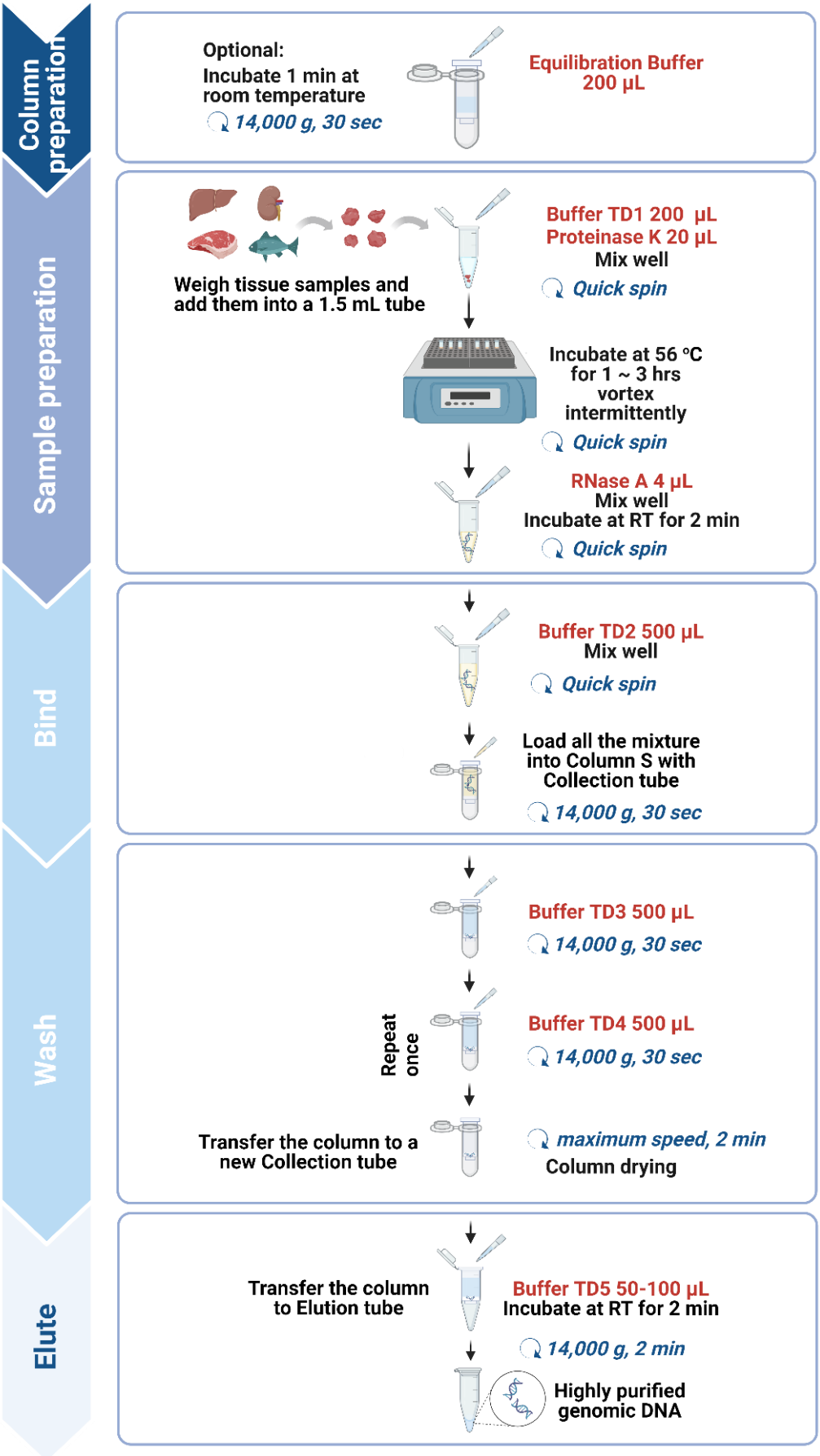
7. Elution

Transfer Column S to **Elution tube**. Add **100 µL Buffer TD5** onto the center of the column, 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*** *to increase the 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 1.5 mL tube
Buffer TD1 200 µL
Proteinase K 20 µL
Mix well
Quick spin
Incubate at 56 °C for 1 ~ 3 hrs vortex intermittently
Quick spin
RNase A 4 µL
Mix well
Incubate at RT for 2 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 (Without Lysing Matrix) has been validated extensively for its performance. In our comparison study, DNA was extracted from soft (liver), fibrous (heart), tough (mouse tail and fish gill), and fatty-rich (beef) tissues with SPINeasy® DNA Kit for Tissue (without Lysing Matrix) and Competitor Q kit. The SPINeasy® DNA Kit for Tissue (without Lysing Matr*ix)* gave high DNA yields along with optimal A260/A280 and A260/A230 ratio, indicating a high extraction performance across a wide range of samples.

The SPINeasy® DNA Kit for Tissue (without Lysing Matrix) consistently delivered higher yield compared to Competitor Q kit across all sample types (Figure 1). It also demonstrated better DNA purity as A260/A230 ratio was closer to 2.0 as compared to that of competitor Q kit. The DNA Integrity Number (DIN) were equivalent for samples extracted from both kits.

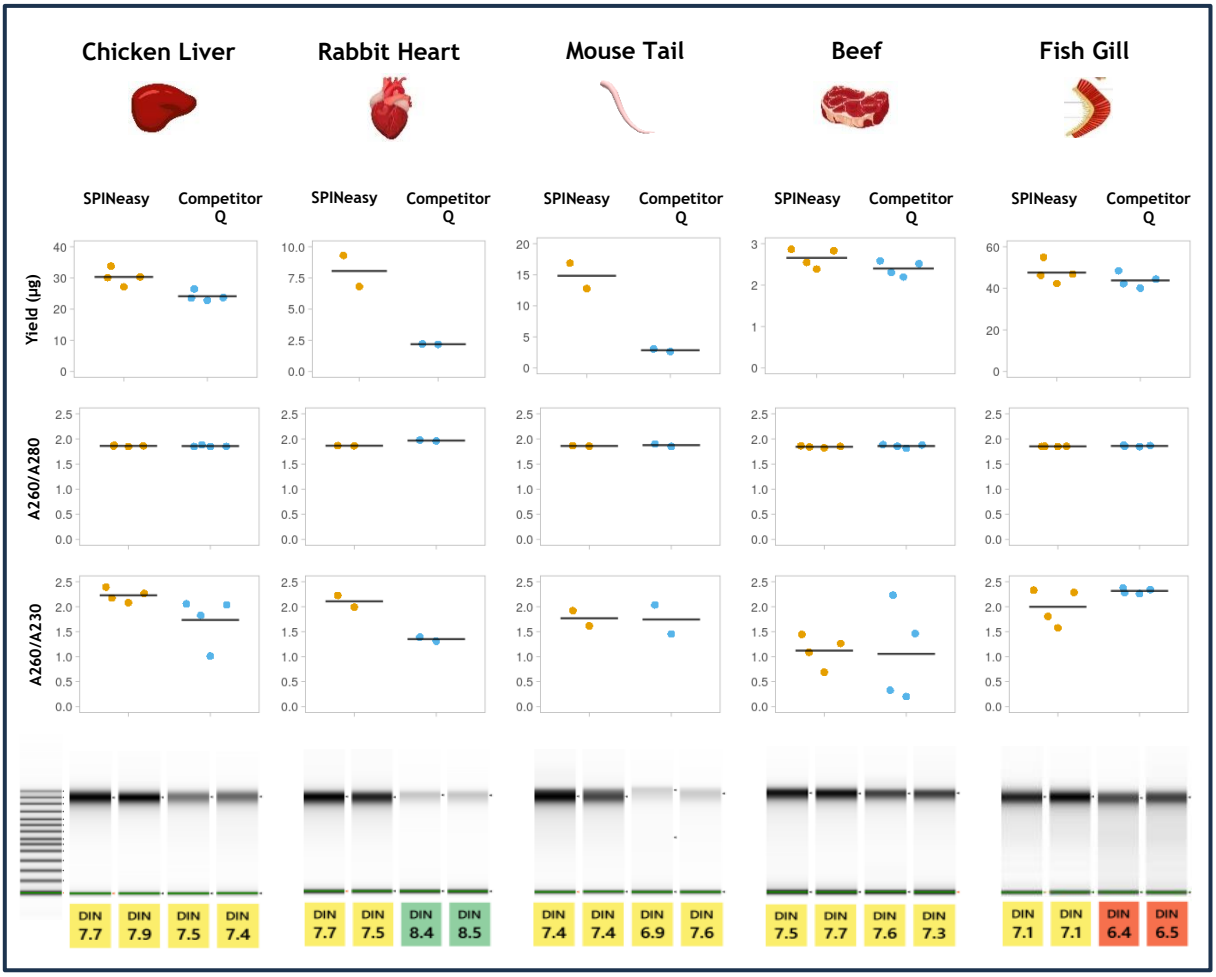


Figure 1: Comparison of quality and quantity of gDNA extracted from various sample types using SPINeasy® DNA Kit for Tissue and Competitor Q. The values of yield, A260/A230 and A260/A280 ratio (from 15 mg of

respective tissues) were measured with a spectrometer. The virtual gel image and DNA Integrity Number (DIN, in green, yellow or red) were analyzed using Agilent Tapestation 4150.

DNA isolated from various rabbit and mouse tissues using the SPINeasy® DNA Kit for Tissue (Without 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 assays (Figure 2, lower panel). The Ct values of gDNA isolated from three different tissues using this kit and Competitor Q were similar, indicating comparable amplifiability.

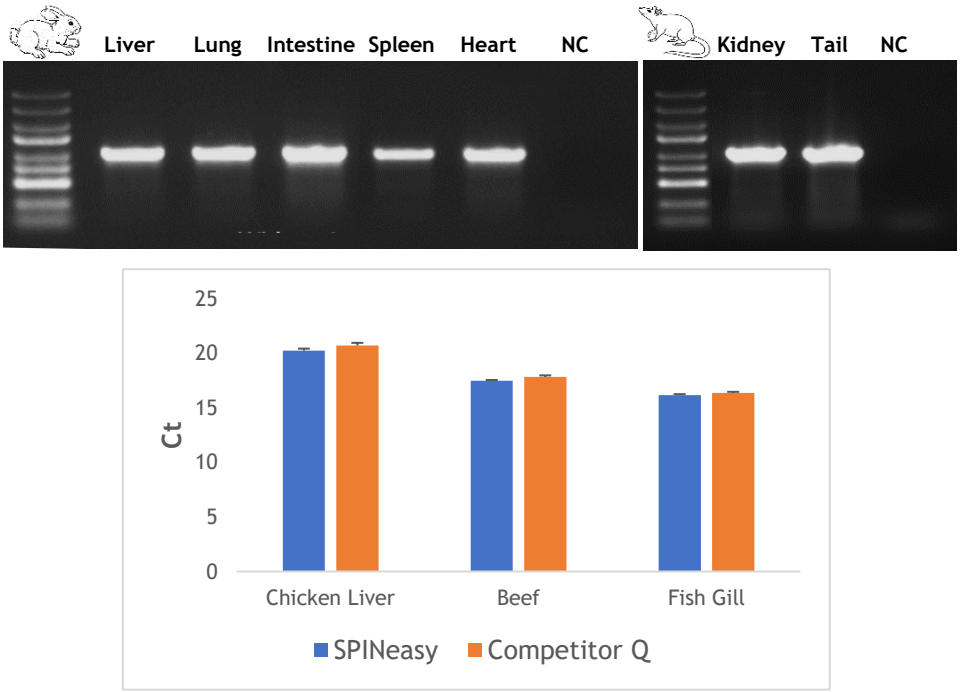


Figure 2: Amplifiability of DNA extracted with SPINeasy® DNA Kit for Tissue (Without Lysing Matrix)

Upper panel: Agarose gel electrophoresis of PCR products amplified from gDNA isolated from various rabbit/mouse tissues using SPINeasy® DNA Kit for Tissue (without 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) extracted with SPINeasy® DNA Kit and Competitor Q. Targets were amplified with SYBR green.

9. Troubleshooting

Problem	Possible Cause	Recommendation
Low DNA Yield / reduced integrity	Insufficient tissue lysis	Some samples may require longer time to complete lysis. Incubation temperature can be increased up to 65°C. Vortex for 15 sec every 20 min can promote tissue digestion.
	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 M 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 A260/280 or A260/230 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	Some samples may require a longer time to complete lysis. Incubation temperature can be increased up to 65 °C. Vortex for 15 sec every 20 min can promote tissue digestion.
	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 the rim of 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	Prolonged incubation will generally result in a compromised DNA integrity.
	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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