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Evaluation Performance

1) Purpose
To evaluate whether residual RNA can be removed through RNase A treatment during DNA extraction using the AllSpin™ Mini Kit, by comparing it with a protocol without RNase A treatment.

2) Sample and kit used
- 10 mg of mouse liver
- Allspin™ mini (#306-150)

3) Tissue DNA extraction protocol with RNase A treatment
1. Harvest tissue samples in a 2.0 ml microcentrifuge tube(not provided).
2. Add 350 µl of Buffer CTL to the tube and disrupt and homogenize the sample by homogenization.
3. Incubate the lysate for 10 min at room temperature.
4. Carefully transfer the supernatant to a Column Type B (red ring) and centrifuge at maximum speed for 3 min at room temperature.
5. Centrifuge at ≥10,000 x g for 30 sec at room temperature. Transfer the mini column to a new 2.0 ml collection tube (provided).
6. Add 20 µl of RNase A (20mg/ml, Cat. No. 391-001, not provided) and incubate at room temperature for 10 min.
7. Add 500 µl of Buffer BW to the mini column.
8. Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.
9. Add 500 µl of Buffer RNW to the mini column.
10. Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.
11. Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).
12. Add 100 µl of Buffer AE to the center of the membrane in the mini column. Let it stand for 1 min. According to the expected yield, the volume of eluent can be adjusted.
13. Centrifuge at ≥10,000 x g for 1 min at room temperature.

4) DNA Con and purity assessment by NanoDrop

Target	RNase A	Conc. (ng/µl)	Yield (µg)	A_{260}/A_{280}	A_{260}/A_{230}
DNA	O	143.1	14.3	1.86	2.38
		102.2	10.2	1.84	2.43
		102.9	10.3	1.86	1.86
	Average	**116.1**	**11.6**	**1.85**	**2.22**
	CV	**0.20**	**0.20**	**0.01**	**0.14**
	X	95.7	9.6	1.84	2.38
		160.3	16.0	1.88	2.23
		98.2	9.8	1.88	2.41
	Average	**118.1**	**11.8**	**1.87**	**2.34**
	CV	**0.31**	**0.31**	**0.01**	**0.04**

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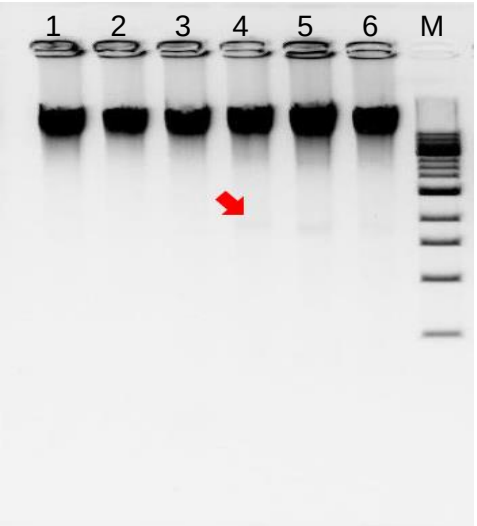
5) DNA integrity assessment



Lane 1-3: RNase-A treated DNA Extraction

Lane 4-5: Original DNA extraction protocol without RNase-A treatment

M: GENESTA™ 1Kb DNA Ladder

6) Conclusion

- Due to the nature of the AllSpin™ kit, which extracts both DNA and RNA using the same buffer system, the original protocol may result in incomplete removal of RNA from the DNA extraction.
- This evaluation confirms that, if RNA-free DNA is required, a simple one-step RNase A treatment can be effectively applied to obtain pure DNA.