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VIOGENE

Ver.: 01A

User Bulletin

*FFPE DNA/R*NA Extraction Miniprep System

Viogene® ***FFPE* DNA/RNA** Extraction Miniprep System provides a fast method to purify total DNA/RNA from FFPE samples. A simple spin-column based method can isolate total genomic DNA, large RNAs, siRNAs and microRNAs without the time-consuming procedure of phenol/chloroform extraction and ethanol precipitation, the highly purified total DNA/RNA can be used for quantitative detection of DNA/RNA level using quantitative method, such as qPCR, digital PCR or microarray.

Sample Preparation Time: ~ 40 minutes, operation under biosafety hood.

For Research Use Only

Downstream Application

* qPCR
* Digital PCR
* Microarray
* cDNA synthesis
* RT-PCR

Product Contents

Cat. No	FFDR1001	FFDR1002
Preps	50	250
FLYS Buffer	12ml	60ml
FX Buffer	17ml	85ml
WS Buffer (RNA)	16ml	40ml x 2
Proteinase K	10mg	10mg x 5
DNA/RNA Mini Column	50	250
Collection Tube	50	250
Protocol	1	1

Shipping & Storage

Viogene FFPE DNA/ RNA Extraction Miniprep System is stable at 20-25°C for one year. The kit should be stored in a dry place and kept away from direct sunlight.

Must-read Notes:

Please read the following notes before starting the procedures.

- Add 64 ml/bt (for FFDR1001) or 160 ml/bt (for FFDR1002) of 98-100% ethanol into WS Buffer bottle when first open.
- Add 1 ml sterile ddH_2O to reconstitute the provided Proteinase K by vortexing. Store the solution at 4 ℃.
- Buffers provided in this system contain irritants. Appropriate safety apparels such as gloves and lab coat should be worn.
- All plastic wares and containers should be treated properly to make sure RNase-free. Gloves should be worn when handling RNA.
- All centrifugation steps except cell pelleting, should be at full speed (10,000 *x g* or 13,000 - 14,000 rpm) in a microcentrifuge.
- All procedure should be done at room temperature (20-25°C).

Viogene's unique design — EasyLid™

The EasyLid™ is designed to prevent contamination during the procedure.

Tips for EasyLid™ —

Twist the arm of the cap and pull the cap to break the EasyLid™.

Protocol:

Buffer Preparation

Add 64 ml/bt (for FFDR1001) or 160 ml/bt (for FFDR1002) of 98-100% ethanol into WS Buffer bottle when first open.

Sample Preparation

1. **Use a scalpel to shave the tissue block in small pieces and trim the excess paraffin. Collect the sample into a 1.5-ml sterile tube.**
   If the sample surface has been exposed to air, discard the first 2–3 sections. Do not use more than 60 mg of FFPE tissue per reaction and cut the sample as thin as possible.

Deparafinization

2. **Add 1 ml xylene, vortex 10-20 sec, incubate at room temperature (RT) for 5-10 min (*vortexing every 2-3 min*) and centrifuge at full speed for 2 min, remove the supernatant by pipetting carefully.**
   If the sample is too thick or more than 30 mg, repeat this procedure once to thoroughly deparaffinize; the completely deparafinized sample should be clear without white deposition.

3. **Wash the sample twice with 1 ml ethanol (98-100%) by vortexing 10 sec and centrifuging at full speed for 1-2 min, and remove the supernatant by pipetting carefully.**

4. **Keep the cap of the tube open, and stand the washed sample in RT for at least 10 min to evaporate residual ethanol.**
   The weight of deparafinized sample should be less than 1/5 of FFPE tissue collected in step 1.

Sample lysis

5. **Add 180 µl buffer FLYS to the sample and mix by vortexing**
   If the sample is too thick, use a homogenizer to homogenize the sample.

6. **Add 20 µl Proteinase K (10 mg/ml) to the sample. Mix immediately by vortexing for 20 sec.**

7. **Incubate at 56-60 °C for 1 hour to lyse the sample. Vortex or invert the sample every 5-10 min.**
   The complete lysed sample should appear clearly, if solid sample still appears, add another **10 µl Proteinase K** to the sample and extend the incubation time to another hour for complete lysis.

8. **Incubate the Proteinase K treated sample at 90 °C for 15 min to inactivate the Proteinase K.**
   If only one heating block can be applied, keep the sample at RT until the temperature reaches 90°C then place the sample onto the heating block.

9. **Centrifuge at full speed for 2 min and transfer the supernatant to a new 2 ml tube.**

10. **Add 300 µl of FX Buffer to the sample, and mix by vortexing.**

11. **Meanwhile, preheat ddH_2O or TE buffer at 70 °C for DNA elution.**

Nucleic acid purification

12. **Add 200 µl of isopropanol to the sample and mix by vortexing.**
    If the sample mixture is more than 550 µl, increase the amount of ethanol proportionally.

13. **Place a DNA/RNA Mini Column onto a Collection Tube. Pipet all the mixture (including any precipitate) into the column without touching the rim. Centrifuge at full speed for 2 minutes. Place the column onto a new Collection Tube.**
    If a precipitate formed from step 12, apply the precipitate and mixture into the DNA/RNA Mini Column.
    If the DNA/RNA Mini Column is clogging after 2 minutes spin, centrifuge again at full speed for another 2 minutes to complete the sample loading.

14. **Wash twice of the column/collection tube with 700 µl WS buffer (ethanol added) by centrifugation at full speed for 30-60 sec, discard the flow-through.**

15. **To completely remove the residual washing buffer and ethanol, centrifuge the column for an additional 5 minutes.**

16. **Transfer the column carefully into a DNase/RNase-free tube (not provided). Add 200 µl of the preheated elution solution of Step 11 to the column. Stand the column for 3-5 minutes, and centrifuge for 1-2 minutes to elute DNA/RNA.**

17. **Store eluted DNA at 4 °C or –20 °C.**

Troubleshooting

Observation	Possible cause	Comments/suggestions
Low or no DNA/RNA yield	Inefficient lysis of sample	Ensure that the sample is completely deparafinized. If not, repeat xylene wash one more time. To evaporate residual ethanol after deparafinization. Use fresh Proteinase K, extend the digestion time, and make sure that the digestion temperature is lower than 60℃. Use sufficient starting materials as suggested. Decrease it, if more than suggested amount of the sample used.
	Insufficient sample storage	Storage samples at -20˚C or colder -70˚C/liquid nitrogen. Keep samples freezed until DNA/RNA extraction, avoiding samples freeze and thaw. If problem persist, use fresh samples and process immediately.
	Column clogging	Too much starting materials used. Decrease the samples to suggested sample amount. Check lysate for any tissues or particle remaining. Remove particles by centrifuge for 5 minutes/ 10,000xg and transfer supernatant to a new tube. Make sure that completes deparafinization and disruption of samples. Use sufficient starting materials as suggested. Decrease it, if more than suggested amount of the sample used Centrifugation temperature too low. The centrifugation temperature should be 20–25˚C.
Poor performance of DNA/RNA in downstream application	Fragmented DNA/RNA	Keep the amplicons as short time as possible.
	Ethanol or salt carryover	Centrifuge the DNA/RNA mini column at full speed for 5 minutes to completely dry the membrane. Store buffer WS at RT, and ensure that the buffers are mixed thoroughly before use.

Serial Products

Product Name	Cat. No.	Preps
miTotal™ RNA Miniprep	VTR1001	50
	VTR1002	250
FFPE miTotal™ RNA Miniprep	FFVTR1001	50
	FFVTR1002	250