Version 2.2

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Storage:	Enzyme Mix stored at –20°C All other components stored at 2-8°C

Shelf Life

Storage options	Shelf life
All components stored at 2-8°C	6 months
Enzyme Mix stored at –20°C All other components stored at 2-8°C	1 year

For research use only, not for use in diagnostic procedures

Limited Product Warranty

It is imperative that the users strictly adhere to this manual. Failure to do so will void TrimGen's guarantee of this product. TrimGen Corporation makes no other warranties of any kind, expressed or implied, including without limitation, warranties of merchantability or fitness for a particular purpose.

Notice to Purchaser

The product is provided as “Research Use Only, Not for use in diagnostic procedures”. The purchaser must determine the suitability of the product for their particular use.

The purchase of Waxfree™ DNA kit includes a limited, nonexclusive license to use the kit. This license does not grant rights to reproduce or modify the Waxfree™ DNA kit for resale, or to use the Waxfree™ DNA kit to manufacture commercial products without written approval of TrimGen Corporation. No other license, expressed, implied or by estoppels is granted.

Product Safety and Liabilities

When working with the kit reagents, always wear a suitable lab coat, disposable gloves, and protective goggles. TrimGen Corporation shall not be liable for any direct, indirect, consequential or incidental damages arising out of the misuse, the results of use, or the inability to use this product.

Introduction

DNA in the FFPE samples is poorly degraded during tissue fixation. Most commercial DNA extraction kits inefficiently bind short DNA fragments making the DNA yield low. The WaxFree kit is specially designed for FFPE samples. The kit uses a special resin to remove PCR inhibitors from the tissue, leaving all of the DNA or RNA in the extract (eliminates DNA loss). The kit has been validated using a variety of FFPE samples. With a simple procedure and high DNA yield, the kit ensures a PCR amplification success rate of > 95%.
WaxFree™ DNA kit apply to:

1. Formalin fixed, paraffin-embedded (FFPE) tissue
2. Fine needle aspiration (FNA) samples
3. Fresh or frozen tissue
4. Cells

The WaxFree-Resin and the enzyme mix are optimized to maximally release DNA from tissues to increase the yield of DNA. The extracted DNA from one paraffin section (size 1 x 1cm, 10μm thickness) is sufficient to perform up to 100 PCR reactions.

The kit uses Q-Solution, a non-toxic solution to efficiently remove paraffin and formalin residual from tissue.

The WaxFree™ DNA kit can be used with the **Standard Protocol** or **Short Protocol**. The applications for each protocol are listed in the table below:

Tissue Type	**Standard Protocol** with deparaffinization	**Short Protocol** without deparaffinization
FFPE section on slides	✓	✓
FFPE sections in tube	✓ Recommended	✕
Tissue dissected from paraffin slide or block	✕	✓
Fine needle aspiration (FNA) sample	✕	✓
Fresh or frozen tissues	✕	✓
Cells	✕	✓

Frequently Asked Questions

Q. How much tissue should I start with?

A: One paraffin section (tissue size 1-2 cm^2 and 5-20μm thick) is sufficient for extraction. For three or more tissue sections, it is necessary to increase the reagent volume and incubation time. The short protocol can start with a very small amount of tissue, such as a Fine Needle Aspiration (FNA) samples.

Q: Can I use short protocol for regular FFPE samples?

A: Yes. The short protocol is simple and works for most FFPE samples.

Q: Can I use the kit to extract DNA from old paraffin samples?

A: Yes. We have used the kit to successfully extract DNA from 10 year-old paraffin samples and the PCR results were excellent.

Q: Can I use the kit to extract RNA?

A: No. The kit reagents are not designed for RNA. A kit designed for RNA is available (WaxFree RNA™ kit Cat No. WR-50).

Q. Can I use the extracted DNA for methylation study?

A: Yes. You can directly treat the final extract with bisulfate and then perform methylation PCR.

Q. Can I use the extracted DNA for a microarray study?

A: Yes. The final extract of WaxFree DNA™ can be used for microarray studies. Further purification may be necessary depending on sample quality.

Q. Can I use WaxFree DNA to extract and amplify 500bp PCR products?

A: Yes. Our customers have successfully amplified 534bp products from 10-year-old FFPE samples and 700bp products

from a 3-year-old FFPE sample. The sample size is determined by the fixation method and the initial quality of the sample.

Kit Contents

Component	WF-50 50 extractions	WF-100 100 extractions
Q-Solution	50 ml	100 ml
Wash Buffer	60 ml	120 ml
Enzyme Mix	0.5 ml	0.9 ml
WaxFree™ Resin	7.5 ml	15 ml
WR-Filter	50	100

Materials and Equipment Needed

2 ml screw-cap microcentrifuge tubes
Laboratory incubator
Heat Block
Vortex Mixer
Microcentrifuge

Standard Protocol

Apply to
- Paraffin section on a slide
- Paraffin sections in tube
- Paraffin tissue from a paraffin block

Pre-heat two incubators or heat blocks, set one at 55°C and the other at 95°C.

1. Collect 1.5 or 2 ml tubes (screw cap) and label the tubes with sample ID.
2. Collect sample

   Paraffin section on slide: one section with tissue size 1-2 cm^2 and 5-20 µm thickness. Scrape the tissue from slide and transfer to a tube.

   Paraffin section prepared in tube: go to step 3.

Paraffin tissue block: Trim away surrounding paraffin. Cut and transfer 10-30mg of tissue to a tube.

3. Add **0.8 ml of Q-Solution** to each tube. Screw cap on and vortex for 30 seconds at high speed.

   Blank control:
   If you are planning to measure the DNA concentration after the extraction, you need to set up a **Blank Control** tube. Add an extra tube to Step 3 and process it as a sample, but without adding any tissue. This tube is the **Blank Control** for $OD_{260/280}$ calibration.

4. Incubate the tube at 55°C for 20 minutes.
5. Vortex the tube 30 seconds at high speed.
6. Centrifuge the tube at 10,000 x *g* (about 12,000-14,000 rpm in most tabletop centrifuge) for 10 minutes.
7. Discard the supernatant using a pipettor or by aspiration. Be careful not to disturb the tissue pellet.

8. Add 1 ml of **Wash Buffer** to the tube.

9. Screw cap on and **vortex 10 seconds** at high speed to re-suspend the pellet.

10. **Centrifuge the tube at 10,000 x *g*** (about 12,000-14,000 rpm in most tabletop centrifuge) for 10 minutes.

11. **Discard the supernatant** using a pipettor or by aspiration. Be careful not to disturb the pellet.

12. Re-suspend **WaxFree™ Resin** by shaking the bottle several times. **Transfer 120µl of the WaxFree™ Resin** to each tube.

13. Add 7µl of **Enzyme Mix** to each tube. Screw cap on and mix the content by flicking the tube.

Return Enzyme Mix to -20°C for storage.

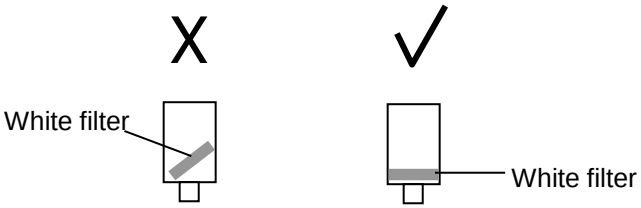
> **For multiple samples**
>
> Pre-mix the **WaxFree™ Resin** with **Enzyme Mix** using the convenient calculation table (see Table 2 on page 16).
>
> Transfer 127µl of the mixture to each tube.

14. Incubate the tube at 55°C for 1 hour.

    One hour incubation is sufficient to extract DNA for regular PCR application. If you need more DNA, a longer incubation time (3 hours or overnight) will increase DNA yield. For skin or muscle tissue, incubate the tube overnight.

15. Heat the tube at 95°C for 10 minutes.

16. Place **WR-Filter** into a new tube and label the tube with sample ID. Make sure the white filter is at the bottom of the column (see below)



17. After incubation, transfer entire extraction mix to the **WR-filter**. Centrifuge at 1,000 x *g* (about 1500-3,000 rpm in most tabletop centrifuge) for 2-3 minutes.

18. Discard the **WR-Filter**. The solution in the tube is the final extract which contains DNA. The extract is ready for PCR amplification.

    The WR-filter removes undigested tissue and WaxFree resin. After the final spin, the final extract may look cloudy, however, it will not affect the PCR reaction.

19. Storage of extracted DNA: *see page 12*

20. DNA Concentration Measurement: *see page 12.*

21. PCR amplification: *see page 13.*

Short Protocol

Apply to
- Paraffin section on slide
- Paraffin tissue block
- Fine needle aspiration (FNA) sample
- Fresh tissue
- Frozen tissue
- Cells

Pre-heat two incubators or heat blocks, set one at 55°C and the other at 95°C.

1. Collect 1.5 or 2 ml tubes (screw cap) and label the tubes with sample ID.

2. Collect sample

   Paraffin section on slide: One section with tissue size 1-2 cm^2 and 5-20µm thicknesses. Scrape the tissue from slide and transfer to a tube.

   Paraffin tissue block: Trim away surrounding paraffin. Cut and transfer 10-30 mg of tissue to a tube.

   Fine needle aspiration sample: Transfer **entire sample** to a tube.

   Fresh or frozen tissue: Cut and transfer **10-30mg of tissue** to a tube.

   Culture cells: Transfer 50µl cell suspension (**10^3-10^8 cells** in PBS) to a tube.

Blank control:
If you are planning to measure the DNA concentration after the extraction, you need to set up a **Blank Control** tube. Add an extra tube to Step 3 and process it as a sample, but without adding any tissue. This tube is the **Blank Control** for $OD_{260/280}$ calibration.

3. Re-suspend the **WaxFree™ Resin** by shaking the bottle several times. Transfer 120μl (for small size tissue add 60μl, see table 1) of the **WaxFree™ Resin** into each tube.

4. Add 7μl (for small size tissue add 3.5μl, see table 1) of **Enzyme Mix** to each tube.

   Return the Enzyme Mix to -20°C

Table 1

Tissues Size	WaxFree-Resin (μl)	Enzyme Mix (μl)
FFPE on slide 0.5-2 cm^2	120	7
FFPE on slide < 0.5 cm^2	60	3.5
FNA	60	3.5
Fresh tissue 10-30 mg	120	7
Fresh tissue < 10 mg	60	3.5
Cells > 10^5 – 10^8	120	7
Cell < 10^5	60	3.5

For multiple samples

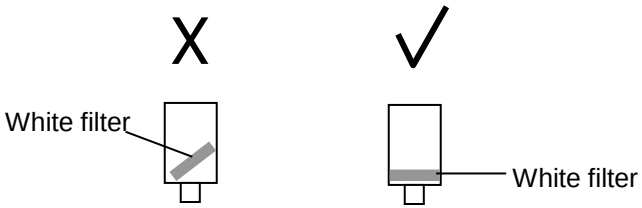
Pre-mix the **WaxFree™ Resin** with **Enzyme Mix** using the convenient calculation table (see Table 2 on page 16).

Transfer 127μl (63μl for small size tissue) of the mixture to each tube.

5. Screw cap on and mix the content by flicking the tube.
6. Incubate the tube at 55°C for 1 hour.

   One hour incubation is sufficient to extract DNA for regular PCR application. If you need more DNA, a longer incubation time (3 hours or overnight) will increase DNA yield. For skin or muscle tissue, incubate the tube overnight.

7. Heat the tube at 95°C for 10 minutes.
8. Place **WR-Filter** into 1.5 ml tubes and label the tubes with sample ID. Make sure the white filter is at the bottom of the column (see below):



9. After incubation, transfer the entire extraction mix to the **WR-filter**.
10. Centrifuge at 1,000 x *g* (about 1,500-3,000 rpm in most tabletop centrifuge) for 2-3 minutes.
11. Discard the **WR-Filter**. The solution in the tube is the final extract that contains DNA and ready for PCR amplification.

    The WR-filter removes undigested tissue and WaxFree resin. After the final spin, the final extract may look cloudy but this will not affect the PCR reaction.

12. Storage of extracted DNA: *see page 12*
13. DNA Concentration Measurement: *see page 12.*
14. PCR amplification: *see page 13.*

DNA Storage

The extracted DNA can be **stored at -20°C**.

DNA Concentration Measurement

Measure by OD_{260} method

Aliquot 5 μl of the final extract to a new tube and dilute the final extract with 45 μl water.

Calibrate the UV spectrophotometer using the diluted **Blank Control** and adjust the OD_{260} and OD_{280} to zero to remove any background absorbance caused by the reagents. Then measure the diluted samples at $OD_{260/280}$ to calculate the DNA concentration.

The following equation can be used to determine the concentration of the extracted DNA.

DNA Conc. (ng/μl) = (62.9 x OD_{260} – 36 x OD_{280}) x dilution factor /1.5

A convenient calculation form is available online:

www.trimgen.com/wf/DNA-calculation.xls

Measure by fluorescent method

The DNA concentration can be accurately measured by PicoGreen® method:
http://probes.invitrogen.com/media/pis/mp07581.pdf

DNA Quality

WaxFree™ DNA kit uses a homogeneous extraction method. The DNA quality assessment is different from conventional extraction methods. The proteins from tissue remain in the final extraction and will reduce the OD ratio. A typical $OD_{260/280}$ ratio for the final extract ranges from *0.8 to 1.3*. The low OD ratio will not affect the PCR.

PCR Amplification

The PCR enzyme and condition varies in different laboratories. Before starting routine operation, we recommend that the first time user performs a sample titration test to find a proper sample amount for your PCR amplification. As an example, use 0.5μl, 1μl, 2μl of final extracts for a 25μl PCR reaction to determine the best sample volume for PCR.

Regular PCR

Use 0.5-1μl of the final extract for a 25-50 μl PCR reaction.

Real-time PCR

Use 0.2-1μl* of the final extract for a 25-50μl real-time PCR reaction.

***Using excess final extract may negatively affect the real-time PCR reaction.**

For large size section, solid tissue such as brain and liver, the final extract contains a high concentration of DNA. It is necessary to dilute the final extract 2-3 times with nuclease-free water before PCR.

A specially designed PCR master mix optimized for FFPE samples is available at TrimGen (Master Mix, Cat No. GR048).

Troubleshooting Guide

Problem	Suggestions
The removal of paraffin is incomplete	**Too much tissue sample** WaxFree™ DNA standard protocol is optimized for a maximum of 2 FFPE sections (5-20 μm thick, up to a 2 cm^2 each). Reduce the number of sections. Add more Q-solution for de-paraffinization.
The final DNA extract shows a yellow or brown color and the PCR does not work well	**Too much tissue** Dilute the final extract 2-3 times with water. Samples from bone marrow, spleen, and liver contain **high levels of blood components**. The hemoglobin in the sample is the cause of the color and it is also a strong inhibitor of PCR. For these samples, dilution of the final extract may improve the PCR efficiency. It may be necessary to remove these blood components by using a TrimGen spin column (Cat No. TC-100) or another commercially available DNA purification kit.
The $OD_{260/280}$ ratio is below our QC criteria, can I use the extracted DNA for PCR?	**Yes.** The DNA quality assessment is different from conventional extraction methods. A typical $OD_{260/280}$ ratio for the WaxFree™ ranges is *0.6 - 1.0*. **The low OD ratio will not affect the PCR amplification**.
The OD_{260} is too high	**Need Blank Control tube** Use the Blank control tube to calibrate the spectrophotometer, and then measure the OD.

	For larger size tissue sections, or solid tissues, the final DNA extract needs to be diluted with water before measure OD and PCR.
The DNA concentration calculated from the $OD_{260/280}$ reading is low and the PCR does not amplify properly	**The DNA concentration is low** Add more the final extract to the PCR reaction. However, the excess of final extract may inhibit the PCR reaction. It is necessary to titrate the final extract for the PCR reaction. **Less DNA released** For some tissues such as skin and muscle, increasing the enzyme digestion time to 3 hours or overnight at 55°C will increase DNA yield.
The DNA concentration calculated from the $OD_{260/280}$ reading is high and PCR does not work.	**DNA concentration is too high** Dilute the extraction supernatant with nuclease- free water then perform the PCR. **PCR amplicon size is too big** Formalin fixation damages DNA. When the designed amplicon size is too big, the PCR may not work because the genome DNA in the FFPE tissue is already broken. The DNA quality depends on the tissue type, storage time, and fixation conditions. Our customers have successfully amplified 534 bp PCR products from 10-year-old samples, and 700 bp PCR products from 3-year-old samples.

Table 2 Calculation Form for WaxFree Resin and Enzyme Mix

	Large size tissue Mix the reagents and transfer **127 µl** to each tube		Small size tissue Mix the reagents and transfer **63 µl** to each tube	
Sample #	**WaxFree Resin**	**Enzyme Mix**	**WaxFree Resin**	**Enzyme Mix**
3	**432**	**25**	**216**	**13**
4	**576**	**34**	**288**	**17**
5	**720**	**42**	**360**	**21**
6	**864**	**50**	**432**	**25**
7	**1008**	**59**	**504**	**29**
8	**1152**	**67**	**576**	**34**
9	**1296**	**76**	**648**	**38**
10	**1440**	**84**	**720**	**42**
11	**1584**	**92**	**792**	**46**
12	**1728**	**101**	**864**	**50**
13	**1872**	**109**	**936**	**55**
14	**2016**	**118**	**1008**	**59**
15	**2160**	**126**	**1080**	**63**
16	**2304**	**134**	**1152**	**67**
17	**2448**	**143**	**1224**	**71**
18	**2592**	**151**	**1296**	**76**
19	**2736**	**160**	**1368**	**80**
20	**2880**	**168**	**1440**	**84**

Formula for calculation

WaxFree Resin = Sample # x 120 (60 for small tissue) x 1.2 (pipetting error)

Enzyme Mix = Sample # x 7 (3.5 for small tissue) x 1.2 (pipetting error)