TECHNICAL MANUAL

Maxwell® HT DNA FFPE Isolation System

Instructions for use of Product
A6372

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TM539

Maxwell® HT DNA FFPE Isolation System

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Maxwell® HT DNA FFPE Isolation System provides a simple and reliable method for the rapid isolation of genomic DNA (gDNA) from FFPE (formalin-fixed, paraffin-embedded) tissue samples in a multiwell format using an automated liquid handling platform. The purified DNA can be used directly in a variety of downstream applications, including PCR and next-generation sequencing.

The Maxwell® HT DNA FFPE Isolation System purifies nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA.

The kit is configured specifically for high-throughput applications and instruments. Reagents are packaged and provided to support the volume requirements of a wide range of liquid handlers and deck configurations.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® HT DNA FFPE Isolation System	**1 each**	**A6372**

For in vitro Research Use Only.

Each kit contains sufficient reagents for four 96-well plates. Includes:

- 140ml Mineral Oil
- 30ml Proteinase K (PK) Solution
- 110ml Lysis Buffer (LBA)
- 33ml FFPE Resin
- 125ml Buffer A (BWA)
- 2 × 58.8ml Buffer B (BWB)
- 24ml RNase A Solution
- 150ml Nuclease-Free Water

Storage Conditions: Store all Maxwell® HT DNA FFPE Isolation System components at room temperature (15–30°C). Do not refrigerate or freeze any of the reagents.

Safety Information: The Maxwell® HT DNA FFPE Isolation System is designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances used with this system. Ethanol and isopropanol should be considered flammable. Please safely handle and dispose of the liquid wastes generated by this product in accordance with your institutional policies.

3. Before You Begin

The Maxwell® HT DNA FFPE Isolation System performance has been evaluated by isolating DNA from mammalian (mouse and human) FFPE tissue samples with a size range of 0.1mm^3 to 2.0mm^3.

Times, speeds and temperatures in the isolation protocol may need to be optimized for the specific hardware used during implementation.

Kit components are dispensed with enough volume to include trough dead volumes. Excess reagent must be dispensed into an automation trough in order to ensure there is sufficient volume during a run. The residual reagent in a trough may be recovered into a clean, capped tube and used again for the next run.

Thoroughly resuspend the FFPE Resin before use by shaking the bottle vigorously. Turn the resin bottle upside down to ensure that no clumps of resin remain at the bottom of the bottle.

Be sure to turn on the heater, either as part of the method or separately from the method, so that it reaches the set temperature prior to the heated steps in the method. The heater set temperature and the temperature of the liquid inside the well may differ by 10–15°C. If necessary, adjust temperature or calibration settings for the heater control unit to ensure that the proper temperatures within the well are reached. At the appropriate calibration and temperature settings, the surface of the Heat Block Adaptor will be slightly lower than the heater set temperature when measured in the center of the Heat Block Adaptor.

Use aerosol-resistant filtered pipette tips to minimize cross-contamination.

3.A. Materials to be Supplied by the User

Reagents

- 80% ethanol (300µl per sample, plus sufficient volume to cover the bottom of the automation trough)

  Note: Prepare by diluting 95–100% USP/ACS- or molecular biology-grade ethanol with molecular biology-grade water. Using denatured ethanol that contains methanol or isopropanol may decrease DNA purity or yield.
- 100% ethanol (358µl per sample, plus sufficient volume to cover the bottom of the automation trough)
- 100% isopropanol (200µl per sample, plus sufficient volume to cover the bottom of the automation trough)
- 95% ethanol (2 × 100ml, for premixing in each Buffer B bottle before use)

Shaking and Heating Options

This protocol requires the use of a liquid handler with integrated shaking, heating and SLAS plate-moving capabilities. Please contact Promega to discuss hardware configuration options. The following hardware was used during development:

- integrated shaker (Tecan–Te Shake with 3mm orbit)
- heat block (Torrey Pines EchoTherm™ RIC20)
- heat block adapter for Nunc 2.0ml Deep Well Plates

Automation of this chemistry with other integrated heater shaker units or independent heaters and shakers may be possible but will require performance testing during method development.

Supplies and Equipment Options

- 2 × 2.2ml Square-Well Deep Well Plate (for sample waste; Cat.# V6781)
- 1 × Nunc 2.0ml Deep Well Plates (sample processing plate; Cat.# AS9307)
- MagnaBot® 96 Flex Magnetic Separation Device (Cat.# AX5600)

  Contact Promega for pricing and availability.
- elution plate (customer-specified)

4. Description of the Maxwell® HT 96 DNA FFPE Isolation System Protocol

The following protocol describes the purification of genomic DNA from FFPE samples. These steps can be used to develop an automated method. Please contact Promega to inquire about a product evaluation. Find additional high-throughput DNA purification product information at:
www.promega.com/products/dna-purification-quantitation/high-throughput-dna-purification/
E-mail: **htgenomics@promega.com**

4.A. Preprocessing

Note: Perform the following steps at room temperature unless otherwise noted.

1. Place the FFPE tissue section(s) into a 1.5ml or 2.0ml microcentrifuge tube. If using a slide-mounted tissue section, scrape the section of the slide using a clean razor blade. Tap or centrifuge the tube briefly to collect the sample at the bottom of the tube.

   Notes:

   a. Use 5–10 micron-thick tissue sections ranging from 20mm^2 to 200mm^2 for a total of up to 2.0mm^3.

   b. Use caution when using razor blades to scrape sample from the slide.

2. Place the 1.5ml or 2.0ml microcentrifuge tubes containing FFPE sections onto the deck of the instrument.
3. Dispense 25µl of Proteinase K into each sample tube.
4. Dispense 225µl of Lysis Buffer into each sample tube.
5. Layer 300µl of Mineral Oil into each sample tube on top of the Proteinase K/Lysis Buffer layer.
6. Depending on implementation of the automated method, incubation of FFPE samples can be performed on the liquid handler or manually off the deck of the liquid handler. If manual incubation will be performed, remove sample tubes from the deck and place in heat block for incubation.
7. Heat the sample tubes for 30 minutes at 56°C.
8. Heat the sample tubes for 4 hours at 80°C.

   Note: Incubating at 80°C for 4 hours will provide optimal results. Using shorter or longer incubation times may negatively impact performance. Following incubation, samples can be held at room temperature for up to 16 hours before proceeding to extraction. Storing samples at 4°C after preprocessing is not recommended.

9. If manual incubation was performed, return sample tubes to the instrument deck and continue with extraction.

4.B. Extraction

10. Dispense 30µl of FFPE Resin into the processing plate.
11. Mix 10µl of RNase A into the lower aqueous layer containing lysed sample in the sample tubes.
12. Transfer the lower aqueous layer of the sample plus RNase A from the sample tubes to the processing plate.
13. Dispense 162µl of Buffer A into the processing plate.
14. Dispense 358µl of 100% ethanol into the processing plate.

15. Shake the processing plate at 1300rpm for 11 minutes to bind with tip mixing halfway through the incubation. (Shaking must be sufficient to keep resin in suspension.)

    Note: Shake speeds specified are for shakers with a 3mm orbit. If using a shaker with a different orbit, it will be necessary to optimize the speeds needed in order to effectively mix the samples.

16. Transfer the processing plate to the magnet and magnetize the resin for 75 seconds.
17. Remove waste from the processing plate and transfer to a liquid waste position or the first waste plate.
18. Add 100µl of Buffer A to the processing plate.
19. Shake the processing plate at 1500rpm for 30 seconds.
20. Add 200µl of 100% isopropanol to the processing plate.
21. Shake the processing plate at 1300rpm for 2.5 minutes.
22. Transfer the processing plate to the magnet and magnetize for 75 seconds.
23. Remove the waste from the processing plate and transfer to a liquid waste position or the second waste plate.
24. Repeat the following wash steps 2 times:
    a. Add 300µl of Buffer B to the processing plate.
    b. Shake the processing plate at 1300rpm for 2.5 minutes.
    c. Transfer the processing plate to the magnet and magnetize for 75 seconds.
    d. Remove the waste from the processing plate and transfer to the liquid waste position or the second waste plate.
25. Add 300µl of 80% ethanol to the processing plate.
26. Shake the processing plate at 1300rpm for 2.5 minutes.
27. Transfer the processing plate to the magnet and magnetize for 75 seconds.
28. Remove the waste from the processing plate and transfer to a liquid waste position or the second waste plate.
29. Transfer the processing plate to the shaker and air-dry while shaking at 1300rpm for 20 minutes.
30. Add 70µl of Nuclease-Free Water to the processing plate.
31. Incubate the processing plate for 15 minutes at 80°C while shaking at 1500rpm to elute the DNA.

    Note: If performing extraction with a separate heater and shaker, incubate the plate on the heater for 15 minutes with a 20-second shake at the beginning, in the middle and at the end of elution incubation.

32. Transfer the processing plate to the magnet and magnetize for 75 seconds.
33. Transfer the eluted sample from the processing plate to the elution plate.

    Note: The recovered elution volume in the elution plate at the end of the method may be less than the volume of Nuclease-Free Water added due to evaporation on the heater.

4.C. Post-Purification

Determine that the purified DNA sample yield meets the input requirements for the appropriate downstream assay prior to use in that assay. Kit performance is evaluated based upon the purification of amplifiable DNA. Other means of quantitation, including absorbance or fluorescent dye binding, may not correlate with amplification (1). Absorbance readings for purified FFPE samples may overestimate yield; we recommend using other methods for determining yield (1).

5. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor.
Contact information available at: **www.promega.com.** E-mail: **techserv@promega.com.**

Symptoms	Possible Causes and Comments
Lower than expected concentration of DNA in eluate	The kit is designed for use with FFPE tissue samples. It is not designed for use with non-FFPE tissue samples, samples prepared using alternative fixation conditions or fresh or frozen tissue samples. **Note:** Amplifiable yield for a typical FFPE section will depend on tissue size, cellularity, formalin fixation conditions and sample handling.
	Kit performance is evaluated by isolating DNA from 5–10µm thick FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. The kit is not designed for samples outside of this range. Use sections with an overall tissue volume that falls within this range.
	The kit is not designed for use with tissue samples that have been prepared with fixatives other than 10% neutral buffered formalin. Verify the sample fixation conditions with your sample source or provider.
	The kit is not recommended for use with stained slides or sections. Repeat the purification using an unstained sample.
	Kit performance is validated by assessing yield using an amplification assay. Other means of quantitation, including absorbance or fluorescent dye binding, may not accurately predict the amplifiability of extracted DNA. For best results, we recommend using a qPCR assay or similar assay to assess eluate yield and quality.
Lower than expected quality (e.g., the eluate contains highly fragmented DNA or inhibitors of downstream assays)	The tissue section used for purification may include fragmented DNA due to sample handling or formalin fixation conditions. Repeat the purification using an adjacent section to assess whether the level of fragmentation is inherent to the sample.
	Some amplification-based assays are particularly sensitive to the presence of inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the extracted DNA. It is the user's responsibility to verify the compatibility of this product with the downstream assays.

6. References

1. Bonin, S. *et al.* (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arch.* **457**, 309–17.

7. Related Products

Manual Nucleic Acid Purification Kits

Product	Size	Cat.#
ReliaPrep™ gDNA Tissue Miniprep System	250 preps	A2052
ReliaPrep™ Blood gDNA Miniprep System	250 preps	A5082
ReliaPrep™ FFPE Total RNA Miniprep System	100 reactions	Z1002
ReliaPrep™ FFPE gDNA Miniprep System	100 reactions	A2352

Maxwell® Nucleic Acid Purification Kits

Product	Size	Cat.#
Maxwell® RSC DNA FFPE Kit	48 preps	AS1450
Maxwell® RSC RNA FFPE Kit	48 preps	AS1440
Maxwell® HT 96 gDNA Blood Isolation System	96 preps	A2670
Buffer A (BWA)	1 each	A6371

Instruments

Product	Size	Cat.#
Maxprep™ Liquid Handler with the Maxwell® RSC 48 Instrument	1 each	AS9100