SpermX™ Manual Differential Extraction User Guide

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Part # 21130

SpermX Kit Contents

20 Device Kit

InnoGenomics Technologies (IGT) SpermX Devices:	
SpermX Inner Tubes*	20
SpermX Outer Tubes	60
SpermX Blue Caps	20
SpermX Centrifuge Adapter Rack	2 (24 tubes each)

*containing the matrix membrane

IGT SpermX Solutions:	
Epithelial Digest Solution	1 Clear Bottle (30 mL)
Sperm Digest Solution	1 Clear Bottle (46 mL)

100 Device Kit

IGT SpermX Devices:	
SpermX Inner Tubes	100
SpermX Outer Tubes	300
SpermX Blue Caps	100
SpermX Centrifuge Adapter Rack	2 (24 tubes each)

IGT SpermX Solutions:	
Epithelial Digest Solution	1 Clear Bottle (135 mL)
Sperm Digest Solution	1 Clear Bottle (230 mL)

Equipment:

- SpermX Pliers (Cat# 21130-P)
- SpermX Centrifuge Adapter Rack (Cat# 21130-R)
- Centrifuge compatible with SpermX adapter rack (ex. 5810/R Eppendorf Centrifuge with MTP swing buckets)
- Incubator Oven Capable of reaching 63°C
- ThermoMixer (Optional) (compatible instruments are ThermoMixer R/Comfort and Mixer HC)
- Thermoblock for SpermX outer tubes (compatible with Eppendorf ThermoMixer R/Comfort) (Optional)
- Microcentrifuge Tubes

Note: 20mg/mL Proteinase K and 1M DTT must be provided by the customer. During IGT investigations and validation, Proteinase K was purchased from ThermoFisher Scientific (Cat #25530049). DTT was purchased from VWR (Cat# 97061-340) and dissolved with 18MΩ H_2O to a final concentration of 1M.

Storage Conditions

Upon receipt, store kit at room temperature (~20°C). Sperm Digest Solution must be kept in a dark location when not in use.

IMPORTANT! If solutions have precipitate upon inspection, warm to 37°C for 10 min to dissolve the precipitate back into solution.

SpermX Differential Digest Protocol

PART A. Removal of Cells from Substrate and Primary Epithelial Digest

1) Assemble a SpermX device by seating a SpermX inner tube in the lower position (see figure below) of the SpermX outer tube.


Inner Tube

Outer Tube


Correct Assembly

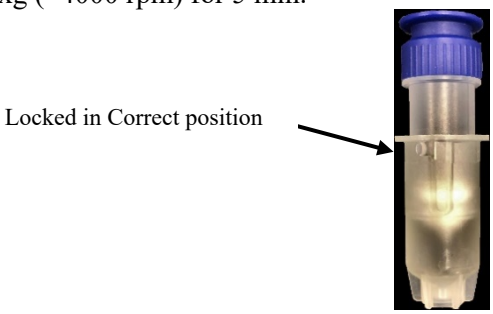
Note: Proper positioning of the SpermX inner tube into the lower position is critical for proper retention of solution and digestion. Incorrect positioning of the SpermX inner tube, not seated all the way to the bottom (gap present), will result in loss of solution, inefficient digestion and poor recoveries!

2) Break or cut an evidentiary swab to remove the intact head from the stick or applicator (i.e. leaving the swab on the stick) and place inside the inner tube. Alternatively, cutting(s) from the evidence material (see note in troubleshooting section for more details) can be placed inside the inner tube.

3) Add 585 µL of Epithelial Digest Solution and 30 µL Proteinase K (20 mg/ml) and cap the SpermX device.

4) Once the SpermX device is assembled with the epithelial digest / pK mixture added, vortex the devices for 10 seconds to ensure thorough mixing. Place the SpermX device into an incubation oven* and incubate at 56°C for 1.5 hr. Vortex the samples for 10 seconds every half hour, allowing for a homogenous solution throughout the digestion period.

5) Raise the SpermX inner tube to the upper position (it is recommended to use the SpermX Pliers) by rotating the SpermX inner tube to the right (clockwise) and ensuring it has locked into place in the groove of the SpermX outer tube. Place the device into a SpermX centrifuge adapter rack, and centrifuge at 3200xg (~4000 rpm) for 5 min.



Note: Ensure the SpermX inner tube is locked into the appropriate position and will not slide back down into the SpermX outer tube. Potential loss of solution and contamination could result if improperly seated.

6) Remove the SpermX device from the centrifuge and twist to the left (counterclockwise) to unlock. Remove the SpermX inner tube, again using the SpermX Pliers, from the SpermX outer tube and place it into a new SpermX outer tube seated in the lower position, setting the SpermX device aside for Part B. **Do not** remove the evidentiary material from the SpermX device. Transfer the solution from the original SpermX outer tube into a microcentrifuge tube and label this as the epithelial fraction (E1).

* If a thermomixer is available, this may also be used with 900 RPM setting. The thermomixer should be tested for the appropriate temperature with a spare assembled SpermX device, 615 µL water to the inner tube in the lower position, and thermometer to ensure proper heating of the device.

PART B. Secondary Epithelial Digest and Washes of SpermX

Secondary Epithelial Digest:

1) Add 600 µL of Epithelial Digest Solution and 15 µL Proteinase K (20mg/ml)* to the SpermX inner tube in the lower position and vortex for 10 secs. Perform a brief centrifuge (1.5 min, ~4000 rpm) and a 10 sec. vortex to ensure Epithelial Digest / pK Mixture is soaked through the matrix membrane of the Sperm X inner tube.
*Note if very high amounts of female DNA are expected, 585 µL Epithelial Digest Solution and 30 µL Proteinase K ((20mg/ml) may be used.

2) Incubate at 56°C for 30 min, with a 10 second vortex of the samples every 15 min. This will be the secondary epithelial digestion.

3) Raise the SpermX inner tube to the upper position, ensuring the SpermX inner tube has locked into place, and place into a SpermX centrifuge adapter rack. Centrifuge at 3200xg (~4000 rpm) for 5 min. Twist to unlock and remove the SpermX inner tube from the SpermX outer tube and place into a sterile DNA-free 15 mL conical tube (not provided) in preparation for the washes **. The solution in the SpermX outer tube can be collected, if desired, as a secondary epithelial fraction (E2).

** 15 mL conical tubes used were from USA Sci. (cat# 1475-0511) which did not allow the SpermX inner tube to slip into the conical tube. Other conical tubes may be used but must first be tested to ensure the SpermX inner tube does not result in falling into the conical tube, resulting in product failure. Alternatively, the SpermX outer tube of the SpermX device used during the 2nd epithelial digest can be used with solution removed after each wash step. However, the potential carryover risk is greater.

SpermX Inner Tube Washes:

4) Add 500 µL of Sperm Digest Solution to the SpermX inner tube cap and vortex the SpermX inner tube and conical assembly for 10 sec to ensure the walls of the SpermX inner tube are covered, and then centrifuge at 3200xg (~4000 rpm) for 5 min. At this point do not remove the SpermX inner tube from the 15 mL conical tube.

5) Repeat Step 4 two additional times for a total of 3 washes. 1.5 mL of Sperm Digest Solution should be at the bottom of the 15 mL conical tube. Optional: Store this solution as the epithelial wash fraction (EW), if necessary.

6) Remove the SpermX inner tube from the 15 mL conical and place into a new SpermX outer tube. Seat it in the lower position.

PART C. Digestion of Sperm Cells and Elution of Sperm DNA from SpermX

1) For each sample, create a Sperm Digest Mixture making sure to account for loss by adding 10%. Be sure to vortex the mixture to ensure it is a homogeneous mixture before adding to the SpermX devices.

Component	Volume per reaction	# of reactions (plus 10%)	Total for Sperm Digest Mixture
Sperm Digest Solution	296 µL		
20mg/mL proteinase K	24 µL		
1M DTT	80 µL		
	400 µL		

2) Add 400 µL of the Sperm Digest Mixture to the SpermX inner tube in the lower position and vortex for 10 sec and perform a short centrifugation (1.5 min, 4000rpm) to ensure the Sperm Digest Mixture reaches all parts of the matrix membrane for effective digestion. If a thermomixer is not being used, repeat the vortex a second time prior to incubation.

3) Incubate at 63°C for 45 min**,** with a 10 second vortex of the samples every 15 min.

4) Once sperm digestion is complete, raise the Sperm X inner tube to the upper position ensuring it has locked into place and put into the SpermX centrifuge adapter rack. Centrifuge at 3200xg (~4000 rpm) for 5 min. Leave eluted solution in the Sperm X outer tube with the Sperm X inner tube in the upper position.

5) Add 150 µL of Sperm Digest Solution to the SpermX inner tube and wait 5 minutes before centrifuging for 5 minutes at 3200xg (~4000 rpm). Transfer the collected eluate to a clean 1.5 mL tube and label as the sperm fraction (S).

Digestion of all fractions has been completed and some or all fractions can proceed to purification. Process the desired fractions following manufacturer's guidelines.

Troubleshooting

Solution is present on the outside of the Sperm X outer tube after an incubation step	The SpermX inner tube was not properly seated into the lower position as the picture displays in Part A. If this occurs, the digestion may not have occurred effectively and re-digestion may need to take place. Furthermore, decontamination of surfaces that came into contact with the Sperm X outer tube should occur as well.
SpermX Pliers not fitting or working properly	Ensure the Plier's Philip head screw is secured but not over tightened. Over tightening can result in difficult operation. Additionally, if the pliers are not seating correctly rotate them 180° and try again.
During centrifugation the Sperm X inner tube fell to the lower position	The device was not properly seated into the lock position as the picture displays in Part A. If this has occurred, raise the Sperm X inner tube to the lock position and fully lock it into place. Repeat the centrifugation for 5 min to recover as much liquid from the device as possible. A new Sperm X outer tube should be used in future steps if possible.
Low Sperm DNA recovery in the S-fraction when larger amounts are expected	During our optimization studies swabs were tested on the stick and cut off the stick. The swabs left on the stick outperformed the cuttings off of the stick. However, both samples gave acceptable results and either method may be used depending on laboratory protocols. Refer to https://innogenomics.com/products/sperm-x/ for additional studies. Another potential issue is inaccurate temperature during the digestion steps. Make sure to test the temperature of your incubation system before performing the digestions. The best way to do this is to use an uncapped fully assembled device with 615µL of water in the Sperm X inner tube. Use a thermometer to read the temperature and adjust as needed to achieve the correct incubation temperature inside the device.
The SpermX inner device has some discoloration (oxidation)	This is a natural phenomenon that has occurred during the manufacturing of the devices. It has no effect on the ability of the device to allow DNA to flow through or on the digestion capabilities of the solutions. Future lots of the devices will be manufactured to reduce these minor aesthetic imperfections.
Volumes used in the protocol don't adhere to downstream purification volume capabilities	If using purification protocols which do not allow for the volumes that are used in the protocol provided, please contact InnoGenomics Technologies for help in resolving the issue.

NOTE: During the development of products for forensic DNA analysis, InnoGenomics Technologies performs developmental validation studies. However, it is the responsibility of the customer laboratory to perform its own analysis and internal validation studies, and develop its own standard operating procedures and interpretation guidelines, to ensure that the products and services it obtains from InnoGenomics Technologies satisfy or will satisfy the applicable guidelines used by the forensic community and are fit for the customer laboratory's human identification applications.

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