Maxwell 16 LEV with DNA IQ Casework Pro Kit

1. Dilute Erase samples to 400ul with TE^{-4} (add 320µl TE^{-4} to the sperm fraction or 50µl TE^{-4} to the epithelial fraction).
2. Add 200ul of the Lysis Buffer provided with the Maxwell kit to each sample.
3. Place reagent cartridges, LEV plungers, and Elution Tubes with 50µl of Elution Buffer± into their proper positions on the Maxwell Cartridge Rack (±for LCN samples that may need further concentration, use water instead of Elution Buffer) as directed in the Maxwell 16 Casework Pro protocol.
4. Add each sample to the first well of each Maxwell cartridge.
5. Place the Maxwell Cartridge Rack into the Maxwell 16 instrument.
6. Start the Maxwell 16.
7. LCN samples may be concentrated using a speed vac.

INSTRUCTIONS AND PROTOCOLS FOR ERASE SPERM ISOLATION KIT 05/11

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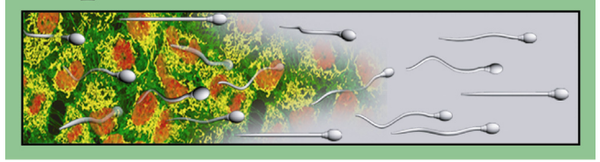
Erase
Sperm Isolation Kit



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INSTRUCTIONS AND PROTOCOLS FOR ERASE SPERM ISOLATION KIT 05/11

Kit Contents

Units	Reagent	Storage
30 ml	Extraction Buffer	4°C or -20°C
2 x 300µl	Proteinase K (Yellow)	-20°C
10 x 100µl	Solution #1 (Red) **MIX BEFORE USE** **DISCARD AFTER USE**	-20°C
10 x 100µl	Solution #2 (White) **PIPETTE MIX ONLY** **DISCARD AFTER USE**	-20°C
10 x 100µl	Solution #3 (Blue) **MIX BEFORE USE** **DISCARD AFTER USE**	-20°C
51	Tube A 2.0 ml Dolphin Tube	Room Temp.
51	Tube B 1.5 ml Pink Tube	Room Temp.
51	Tube C 1.5 ml Blue Tube	Room Temp.
50	Spin Baskets	Room Temp.

Erase Instructions

1. For each sample to be extracted, add 400 µl Extraction Buffer and 7 µl Proteinase K (PK) to a 15 ml conical tube and mix gently to create a master mix.

   Example:
   8 samples x 400 µl = 3200 µl Extraction Buffer, and
   8 samples x 7 µl = 56 µl PK added to 15 ml conical tube

2. Place solid substrate (e.g. cutting, swab, etc.) into Tube A.
3. Pipette 400 µl of the master mix from step 1 into each Tube A* and vortex at full speed for 20 seconds. Pulse centrifuge at less than 6,000xg for 2 seconds to remove liquid from the sides and cap of the tube.
4. Incubate at 56ºC for 1 hour to complete the PK digestion.
5. Pulse centrifuge samples to remove condensation from caps.
6. Using sterile forceps, place the substrate in the provided spin basket; place the basket back in the SAME Tube A.*
7. Cap and centrifuge Tube A at maximum speed for 5 minutes (10,000xg minimum).
8. Remove spin basket from Tube A and discard or retain substrate according to your laboratory's policies.

Dolphin Tube

BE VERY CAREFUL NOT TO DISTURB THE SPERM PELLET.

9. Using the lower line on Tube A as a guide, slowly pipette approximately 330-350 µl of liquid from Tube A into Tube B until approximately 50 µl remains in Tube A. LEAVE APPROX. 50 µl OF EXTRACTION BUFFER OVER THE SPERM PELLET.

10. Tube B is the non-sperm fraction and is ready for DNA purification using your laboratory's preferred method (go to Purification Methods).

11. If desired, the sperm pellet in Tube A may be mixed and a smear made to confirm the presence of spermatozoa.

The order in which solutions are added in the following steps is critical.

12. Add 10 µl of Solution #1 to Tube A.

13. Add 10 µl of Solution #2 to Tube A. Mix by pipetting up and down until the sperm pellet has been fully resuspended. **AVOID BUBBLES AND DO NOT VORTEX. Solution #2 should be mixed thoroughly but gently by pipetting up and down multiple times.**

14. Transfer the liquid to Tube C.**

15. Incubate Tube C samples for 15 minutes at 37 ºC .

16. Thaw and vortex Solution #3 to solubilize any precipitate. Add 10 µl of Solution #3 to Tube C and mix thoroughly or vortex.

17. Incubate Tube C samples at 56ºC for 15 minutes.

18. Tube C is the lysed sperm cell fraction and is ready for DNA purification. (go to Purification Methods).

* *Additional manipulation or agitation of the substrate in the solution at this step may increase the amount of sperm cells released from the substrate.*

** *The tube change at this step is necessary because a trace amount of epithelial DNA adheres to the side of the tube. It may be possible to avoid this tube transfer using the following steps:*

1) *Add Solution #1 and #2 and mix per steps 12 and 13.*
2) *Pipette the solution in Tube A on the inside walls of Tube A up to the 500* µl line. *Be careful to ensure the entire interior tube surface has been rinsed with the solution.*
3) *Pulse centrifuge.*

Purification Methods (choose one)

Phenol Chloroform Isoamyl Alcohol

1. Dilute sample to 450ul with TE^{-4} (add 370 ul TE^{-4} to the sperm fraction or 100 ul TE^{-4} to the epithelial fraction).
2. Add 600ul phenol chloroform isoamyl alcohol.
3. Mix well by shaking sample vigorously.
4. Centrifuge sample for 5 minutes at 12,000xg.
5. Continue to EtOH Precipitation OR Size Filtration

EtOH Precipitation

1. After a phenol chloroform isoamyl procedure, pipette 420 ul of the aqueous layer into a clean 2ml tube.
2. Add 233 ul of 7M NH_4OAc and mix thoroughly.
3. Add 1.3 ml of 100% ethanol and mix thoroughly.
4. Place tube at -80°C for 30 minutes or –20°C for 2 hours.
5. Centrifuge 20 minutes (increased centrifugation time, up to 60 minutes, may benefit LCN samples) at 10,000xg.
6. Gently decant supernatant.
7. Add 300 ul of 70% ethanol and invert tube several times.
8. Centrifuge 20 minutes at 10,000xg.
9. Gently decant supernatant and dry pellet in a vacuum oven.
10. Add desired amount of water or TE-4 and incubate at 37°C for 30 minutes.

Size Filtration (Microcon/Vivacon)

1. After a phenol chloroform isoamyl procedure, pipette the aqueous layer into a new Microcon/Vivacon column.
2. Centrifuge sample for 15 minutes at 2,000xg.
3. Place Microcon/Vivacon column into a clean collection tube or empty the current collection tube.
4. Add 500 ul of TE^{-4} to the column and centrifuge for 15 minutes at 2000xg.
5. Repeat steps 3 & 4 for a total of 3 washes.
6. Add desired elution amount of TE^{-4} or water to the column and invert column into a clean 1.5 ml tube. Centrifuge for 2 minutes at 5000xg.

Qiagen EZ1

1. Dilute sample to 200ul with G2 Buffer if necessary (add 120 ul G2 Buffer to the sperm fraction or use 200 ul of the epithelial fraction).
2. For LCN samples, it may be beneficial to add 1ul PolyA RNA (EZ1 protocol Appendix A, pg . 54).
3. Place reagent trays, tubes and tips into proper EZ1 positions.
4. Set EZ1 for trace protocol, and select the volume and type of elution (choose water for elution of LCN samples).
5. Start the EZ1 machine.
6. LCN samples may be concentrated using a speed vac.