



Application Sheet

Xenographic Retention Chromatography (XRC)

EpiSep[®] Preparation and Isolation of Urothelial Cells from Preserved Urine for use with FISH.

Overview

These are suggested procedures for preparing and isolating urothelial cells from preserved urine. Laboratories should use these as guidelines and optimize the procedure in accordance with their own experience.

WaveSense EpiSep[®] Components

(Research use only)

- Select one from: Anti-Urothelial MagParticles Cat. #R2122-1, R2123-1, R2126-1, R2127-1, R2120-1.
- EpiSep Hybridization Slide[®] (10 Pack) Cat. #A3104-10.
- Magnetic Tube Dock[®] (MTD-50) Cat. #A4101-1
- NeoMag Slide Dock for EpiSep[®] Hybridization Slide (NSD II) Cat. #A1103-1.
- EpiSep 50mL Tube Mixer Cat. #A5101-1
- EpiSep 0.1M TBS Tris Saline pH7.5 Cat. #R2129-1.
- EpiSep Debulking Buffer Cat. #R2137-1

Reagents and Equipment Required (not provided)

- Pipettor (1-100 μ L and 100-1000 μ L)
- Transfer Pipettes (3-5mL)
- 50mL Conical Centrifuge Tube
- Aspirator
- Preserved Urine in (Carbowax, PreservCyt etc.)
- Fixative Solution (CytoLyt, Cytospin, 3:1 Methanol: Acetic acid, PreservCyt etc.)

Magnetic labeling of Urothelial cells from urine using EpiSep Anti-Urothelial[®] Magnetic Particles.

- 1) Transfer preserved urine to the 50mL conical centrifuge tube.
- 2) Centrifuge the specimen(s) at 600xg for 10 minutes at room temperature (15-30°C).
- 3) Aspirate the supernatant to within approximately 0.1mL of the cell precipitate/pellet.
Note: Be careful not to disturb the cell precipitate/pellet.
- 4) Add 1mL of EpiSep Debulking Buffer and mix briefly by hand to re-suspend the cell pellet.
- 5) Add 25-75 μ L of EpiSep Anti-Urothelial Magnetic Particles to the specimen.
- 6) Place the specimen on the EpiSep 50mL Tube Mixer. Turn on the Mixer and incubate the specimen for 10min.
- 7) After 10min, slowly add 1mL of Carnoy's fixative to the specimen while on the EpiSep 50mL Tube Mixer.
- 8) Remove the specimen from the EpiSep 50mL Tube Mixer and place onto the Magnetic Tube Dock[®] (MTD-50[®]) for 5 minutes minimum.

- 9) Swirl MTD-50[®] to be sure that magnetic particles are attached to magnet.

Note: If beads are not visible on the magnet after swirling, remove tube from the MTD-50[®] and re-suspend the sample. Add 1mL of Methanol/Acetic Acid (Carnoy's) fixative and mix. Place tube on Magnetic Tube Dock[®] for 5 minutes. Repeat process until beads remain captured on the magnet and proceed to next step.

Important! It is recommended that the operator move rapidly through steps 10-12 such that following supernatant removal the sample is not allowed to dry. It is recommended that the samples should not be processed in batch at these steps, but rather each sample should be taken through to completion of step 12.

- 10) While on the EpiSep MTD-50[®], remove supernatant.
- 11) Remove 50mL tube from EpiSep MTD-50[®].
- 12) Rinse the magnetic particles from the side of the conical tube using 1.0mL EpiSep 0.1M TBS Tris Saline pH 7.5.

Isolation of magnetically labeled Urothelial Cells from urine using the EpiSep Hybridization Slide[®].

- 1) Label EpiSep Hybridization Slide[®] with specimen(s) identification and place on the NSD II at room temperature (15-30°C).
- 2) Re-suspend specimen using gentle agitation and transfer entirely to the sample well of the EpiSep Hybridization Slide[®]. Wait until the entire sample has absorbed into the slide.
- 3) Rinse the sides of the sample well with 100 μ l of fixative solution. Remove the EpiSep Hybridization Slide[®] absorbent cap and discard.
Note: The magnetic particles with captured cells will appear in the center of the slide over the magnet.
- 4) Allow the slide to air dry on NSD II for 2 to 5 minutes at room temperature (Do not exceed 5 minutes). Remove the slide from the NeoMag Slide Dock[®] and submerge slides in 3:1 Methanol/Glacial Acetic Acid (Carnoy's fixative) at -20°C for 30 minutes or more.
- 5) The slide(s) are now ready for further analysis.



Technical Assistance

For technical assistance, contact the WaveSense, Inc. Technical Service Department at (800) 807-7760 or (949) 341-1980 or Fax to (949) 341-1982.

Customer Service

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