



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.

Reagents Required (not provided)

- 3:1 Methanol/Acetic Acid Fixative.
- Preserved Urine in (Carbowax, PreservCyt etc.)
- 0.1M TRIS Solution.
- Fixative Solution (CytoLyt, Cytospin, 3:1 Methanol: Acetic acid, PerservCyt etc.)

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

- 1) Transfer preserved urine to the 50mL conical centrifuge tube.
- 2) Centrifuge the specimen(s) at 600xg for five to ten minutes at room temperature (15-30°C).
- 3) Remove the supernatant to within approximately 0.5ml (or less) of the cell precipitate/pellet. **Note: Be careful not to disturb the cell precipitate/pellet.**
- 4) Add 1mL of 0.1M TRIS Solution and re-suspend the pellet/precipitate by gentle vortex or aspirate pellet with a transfer pipette.
- 5) Add 25-75 μ L of EpiSep URO-1[®] MagParticles to the sample. Vortex to mix, then incubate for more than 1 minute. **Note: Incubating for 1-15 minutes is optional.**
- 6) Slowly add 4mL of Carnoy's fixative, replace cap and mix to re-suspend cells and particles.
- 7) Place 50mL tube on the Magnetic Tube Dock[®] (MTD-50) for 5 minutes minimum.
- 8) 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 9-11 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 11.

- 9) While on the EpiSep MTD-50[®] remove supernatant.
- 10) Remove 50mL tube from EpiSep MTD-50[®].
- 11) Rinse the magnetic particles from the side of the conical tube using 1.0mL of 0.1M TRIS solution.

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) Add 100 μ l of fixative into the sample well and immediately 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). Removed 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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