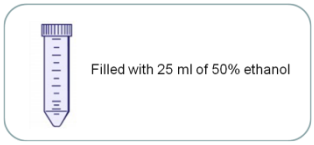
Preparation of Cytology Specimens from Urine:

1. CytoSpin Method/ Ethanol Fixation

1.1 Procedure:

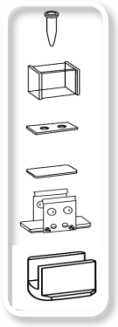


- Collect 25 ml voided urine.
- Fixate over night at 4°C using 25 ml 50% ethanol.

1.2 Sample processing:

- Centrifuge ethanol fixed urine cells for 10 min at 2000 rpm @ RT.
- Discard supernatant.
- Resuspension of cell pellet in 1 ml 1x PBS.

1.3 Slide preparation:



- Mark the clean and dry slide (defatted slide).
- Load slide into the vessel holder.
- Suitable filter card is positioned between the container and the slide.
- Place the micro tubes in the container and pipet 100-200 µl cell suspension in the tubes.
- Centrifuge 10 min at 40xg @ RT.

Due to capillary forces, the sample only enters the container once the centrifugal force is applied. The suspended cells reach the glass surface and stick to it. Excess fluid is absorbed by the filter card.

- Remove the container and the filter card carefully.

Avoid any smearing of the cells!

- Fix the cells immediately by applying fixation spray (Merckofix® Fixationspray).
- Air dry for 10 min.
- Storage of the specimens is possible @ -20°C.

2. Methanol/ Acetic Acid Fixation (Alternative)

2.1 Specimen collection and preparation

- Collect ≥ 33 ml voided urine.
- Mix voided urine 2:1 (v:v) with preservative (e.g. Carbowax or PreservCyt®).

2.2 Sample processing:

- Centrifuge at 600xg for 10 min @ RT in 50 ml centrifuge tube.
- Discard supernatent to approx. 1-2 ml of the cell pellet.
- Resuspend the cell pellet in the remaining 1-2 ml of supernatant.
- Transfer suspension to 15 ml centrifuge tube.
- Centrifuge at 600xg for 10 min @ RT.
- Remove supernatant by remaining 0.5 ml of the cell pellet.
- Resuspend pellet in the remaining 0.5 ml supernatant.
- Slowly add 1-5 ml of fresh fixative (3:1, methanol: acetic acid), dropwise at first, with frequent agitation.
- Let fixed specimens stand for a minimum of 30 min @ -20 °C.
- Centrifuge samples at 600xg for 5 min @ RT.
- Carefully remove supernatant.
- Wash pellet by resuspensing in 1-5 ml fixative (3:1, methanol:acetic acid).
- Centrifuge samples at 600xg for 5 min @ RT.
- Repeat these washing steps twice.
- After centrifugation of the cell pellet in fixative.
- If the cell pellet is hardly visible, carefully remove as much fixative as possible, leaving approx. 100 µl solution.

- If the cell pellet is easily visible, remove as much fixative as possible and add 0.5-1 ml fresh fixative to the cell pellet
- Proceed immediately with slide preparation

2.3 Slide preparation:

- Use 12-well slides.
- Resuspend the cell pellet and apply 3 µl, 10 µl, 30 µl of cell suspension on three slide circles (circle #1, 2, and 3).
- Allow samples to air dry.
- Examine slide under a light microscope using a 20x objective.
- Select the hybridization area (circle #1, 2, and 3) in which 100-200 cells are visible in the field of vision.
- The circle with the best corresponding cell density should be used for hybridization.
- If cell density is too low apply another 30 µl of cell suspension.
- If cell density is too high dilute the cell suspension with fixative and repeat the slide preparation procedure.
- Fixed slides are stable @ -20°C for up to 12 months.

Please proceed with the protocol of the Zyto*Light* FISH Cytology Implementation Kit.