

Urine collection and processing

Specimen Collection

Collect as much urine as possible. 100 mL of urine is optimal but smaller volumes can still be analyzed. Urine should be collected midstream and prior to biopsy or treatment for rejection when applicable. To minimize RNA degradation, urine samples must be kept at 4°C prior to processing and must be processed as soon as possible, but no longer than 4 hours after collection.

Specimen Processing Procedures

Transfer the urine into 50mL Sterile Disposable Centrifuge Tubes. Volumes greater than 50 mL can be split into two or more centrifuge tubes.

Centrifuge the urine at 2,000g at 4°C for 30 minutes. (Please note that you must determine the necessary RPM for 2,000g for your centrifuge.)

Without disturbing the pellet, transfer 1ml aliquots of supernatant to 1.5-mL microcentrifuge polypropylene cryotubes and store at -80°C until shipping.

Pour off the remaining supernatant and invert the tube(s) over absorbent paper to remove all traces of urine.

Resuspend the pellet(s) in 1.0mL of phosphate buffered saline (1X PBS). Make sure that any sediment on the sides of the tube is also collected. (If more than 50 mL of urine was obtained and multiple centrifuge tubes were used, combine the pellet from each tube in a total of 1.0 mL PBS before proceeding to the next step.)

Transfer the resuspended urine pellet to a 1.5 mL microcentrifuge polypropylene tube (one tube per subject).

Centrifuge at 10,000g for 4 minutes at room temperature.

Discard the supernatant gently and completely without disturbing the cell pellet.

Add 150 microliters of RNAlater to the pellet and close the cap tightly. Gently tap the lower portion of the tube with your finger to mix the cell pellet with the RNA later. (Do not try to resuspend the pellet with the pipet tip; it will become clogged.)

Centrifuge the tube at 10,000g for 15 seconds at room temperature to collect all of the liquid at the bottom of the tube.

Store the samples at -80° C.