

Insert Institution Department (if applicable) Division (if applicable) Biorepository (if applicable)	Standard Operating Procedure Collection of Urine with sediment or pellets	
SOP Repository Banking	Blood Processing for Biobank Network Exchange	
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Revision:	Revision Date:	Review Period (insert # yrs)
Authored by:	Approved By:	Date:

1.0 Purpose

This document describes the process for the collection of human urine for storage as raw, supernatants, sediments or pellets.

2.0 Scope

These guidelines apply to personnel intending to cryopreserve urine.

3.0 Requirements:

3.1. General Requirements

All specimens will be treated as potentially hazardous. Personal protective equipment (lab coats, gloves and eye protection) must be worn at all times when handling specimens. Tubes, needles and pipets must be properly disposed of in biohazard containers in accordance with institutional requirements.

3.2. Equipment:

3.2.1 Centrifuge with swinging bucket rotor

3.2.2 -80°C Freezer

3.2.3 Biosafety Cabinet

3.2.4 Pipette Aid

3.2.5 Microcentrifuge

3.3. Materials:

3.3.1 Progensa urine specimen transport tubes

3.3.2 15ml and 50ml Sterile, Polypropylene, Conical, Centrifuge Tubes

3.3.3 Sterile pipettes and/or transfer pipets.

3.3.4 1.5 ml polypropylene microcentrifuge tubes with screw top caps

3.3.5 5 ml storage vials with caps

4.0 Method:

4.1 Urine should be refrigerated or placed on ice after collection.

4.2 Urine should be processed in a biosafety cabinet. Make sure lid is tight on specimen cup; gently invert 5 times to mix; wipe cap with 70% alcohol prior to opening the cup.

4.3 Remove foil seal cap from Progensa tube and place Progensa tubes in a rack (note: tubes contain liquid; keep upright once cap is removed). Transfer 2.5 ml raw (whole) urine to Progensa tubes. Total volume should be between the lines in the 'window' on the tube label. Screw on transfer caps (non-foil); invert 5 times to mix stabilization reagent with raw urine.

4.4 Transfer 5 ml aliquots of raw (whole) urine to 5 ml storage vials.

4.5 If Sediment (pellet) and supernatant is desired:

4.5.1 Transfer 10-30 ml raw urine to a 50 ml conical bottom centrifuge tube.

4.5.2 Centrifuge 10 min, 1000 x g, 4°C, without brake (spin will take about 30 minutes, dependent on centrifuge)

4.5.3 Remove supernatant, leaving behind 50-100 ul. Transfer 5 ml aliquots of supernatant to storage vials, if desired.

4.5.4 If only pellet is needed, resuspend in residual volume and transfer to a 1.5 ml microcentrifuge tube with screw cap for storage. Alternatively, add 5 ml ice cold PBS to pelleted sediment (pellet is loose, and may not be visible). Resuspend and transfer to a 15 ml conical bottom centrifuge tube. Repeat spin (10 min, 1000xg, 4°C, no brake).

4.5.5 Remove and discard supernatant. Add 1 ml ice cold PBS. Resuspend and transfer to a microcentrifuge tube. Spin 10 min, 700xg, 4°C (brake ok).

4.5.6 Remove and discard supernatant. Depending on desired usage for pellet, either resuspend in 250 ul RNeasy lysis buffer or snap freeze the pellet in liquid nitrogen. (If enough urine is available, 2 10-30 ml volumes may be processed; one for RNeasy lysis buffer, and one for snap freezing).

4.6 Check that all aliquot vial caps are secure and that all vials are labeled. Place aliquots upright in specimen box or rack in -80°C or colder freezer. All specimens should remain at -80°C or colder and will be transported frozen on dry ice only.

Record/Data Points (Use Barcode if possible, to facilitate sample tracking)

1. Date and time of urine collection
2. Number and volume of aliquots prepared
3. Date and time urine transferred into -80°C
4. Date and time of shipping (if applicable)
5. Any freeze-thaw that occurs with a sample for any reason
6. Any variations or deviations from the SOP, problems, or issues

Label Cryovials

1. Subject ID
2. Subject initials (if appropriate; may be an identifier)
3. Date of collection

4. Visit date (if applicable; may be an identifier. Visit number may be desired instead.)

Supplies

1. ProgenSA urine specimen transport tubes from Hologics. Catalog #303550
2. Centrifuge with swinging bucket rotor (different times and rcf will be needed for fixed angle rotors)
3. 15 and/or 50 ml polypropylene conical tubes (for example, Corning 430052, Fisher catalog #05-538-53D)
4. 5 ml tubes with caps (for example, Sarstedt 60.558.001)
5. 1.5 ml microcentrifuge tubes with o-ring caps (for example, Fisher cat #50-809-238)
5. 2ml, 5ml, and 10ml pipettes (for example, Fisher cat #13-678-11C, 13-678-11D, 13-678-11E)
6. Disposable transfer pipettes (for example, Fisher cat #13-711-20)
7. Small ice bucket
8. Biohazard waste container suitable for human biologics
9. Appropriately sized racks and freezer storage boxes.
10. Bleach and/or 70% EtOH.