

Standard Protocol for Urine Collection
(developed by the Human Urine and Kidney Proteome Project, HUKPP, and the European Urine and Kidney Proteomics, EuroKUP Initiatives)

1 Type of urine sample

Mid-stream of 2nd morning urine (preferably) or morning random-catch urine, in sterile (preferably) or clean urine collectors.

2. Pre-treatment and storage

Centrifuge at 1000g, for 10 min to remove cell debris and casts. Aliquot supernatant avoiding disturbing the pellets at 1.5, 10, or 50 ml (depending on downstream application); do not overfill the tubes; store at -80°C (preferably) or -20°C. Record time until freezing (it should be no longer than 3hr; see also note 4)

3. Freezing and Thawing

Avoid freeze-thaw cycles. If thawing and re-freezing occurs, always keep a record of this event.

Notes

1. This is a general protocol for urine collection known to provide overall good proteomics data quality for a wide spectrum of technologies and diseases under investigation, based on our accumulated knowledge so far. However it should be noted that for special cases/diseases (eg associated with high proteinuria, hematuria etc) the application of specialized protocols may be recommended. It is likely that these guidelines may change in the future with the collection of new data and knowledge in the field.
2. Sample post-thawing processing steps are not addressed as these largely depend on the technology in use.
3. This protocol is applicable for the analysis of soluble urine proteins. Study of exosomal or other solid phase urine proteins requires the employment of specialized protocols.
4. The addition of preservatives is not in general recommended for spot urine and fast processing times (eg times up to freezing of less than 3hr). For longer times until freezing, storage at 4°C or ice and addition of 10mM NaN₃ (or 0.2M Boric acid) is helpful to inhibit bacterial growth.
5. Results from some studies indicate that the addition of protease inhibitors is not beneficial even though this issue is still under investigation. Therefore, addition of protease inhibitors is not in general considered compulsory for the time being. Study of phosphoproteins requires addition of phosphatase inhibitors.

6. Centrifugation is desirable but not absolutely needed. When centrifugation is not possible, good mixing is recommended prior to aliquoting and recording of whether the sample appeared turbid. If the latter happens most likely the overall sample quality is inferior for proteomics analysis and therefore such samples should be discarded. In all cases, sample processing up to freezing should be performed fairly fast (<3hrs).

7. Monitoring of protein content and other clinical parameters on a urine aliquot should be performed prior to freezing since many of these parameters are altered by freezing. This information should be recorded on the sample sheets. Minimal information required when storing samples is shown below.

8. Freezing results in protein precipitation. For this reason, after thawing of the frozen aliquots, resuspension of the pellets is recommended (for example by pH adjustment to 8.0 by Tris buffer, by mixing with detergents, sonication or else depending on the downstream application) prior to any processing. Centrifugation after freezing should be avoided (particularly for peptidomics analysis), nevertheless this frequently depends on the technology to be employed.

9. The protocol has to be employed in a highly consistent manner. For comparative studies only samples with identical freeze-thaw histories should be employed.

For more information and specialized protocols please visit:

<http://www.hukpp.org> and www.eurokup.org

Minimal Information

A special database with the demographics and clinical data linked to the sample should have been generated by the participating physicians

Minimal information on the sample includes:

A. Storage Information

1. Unique Sample Code
2. Storage temperature (-20/-80):
3. Institution- name of person in charge of collection
4. Date-time of Collection
5. Time until freezing
6. Aliquot (volume) and number of aliquots:

B. Case Information

7. Unique patient code
8. Clinical Diagnosis
9. Age

10. Gender
11. Height
12. Weight

C. Recommended Laboratory Information

13. GFR (or eGFR)
 14. Urinary Protein Amount (and method of calculation)
 15. Urine Creatinine (and method of calculation)
 16. Hematuria
 17. Serum Creatinine* (and method of calculation)
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18. Urine pH*
 19. Serum Protein (serum albumin)* (and method of calculation)
 20. Serum Cholesterol * (and method of calculation)

*not absolutely needed (but usually helpful) information. In general, needed clinical information depends on the disease under investigation