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## UPCK™ Urine Protein Concentration Kit

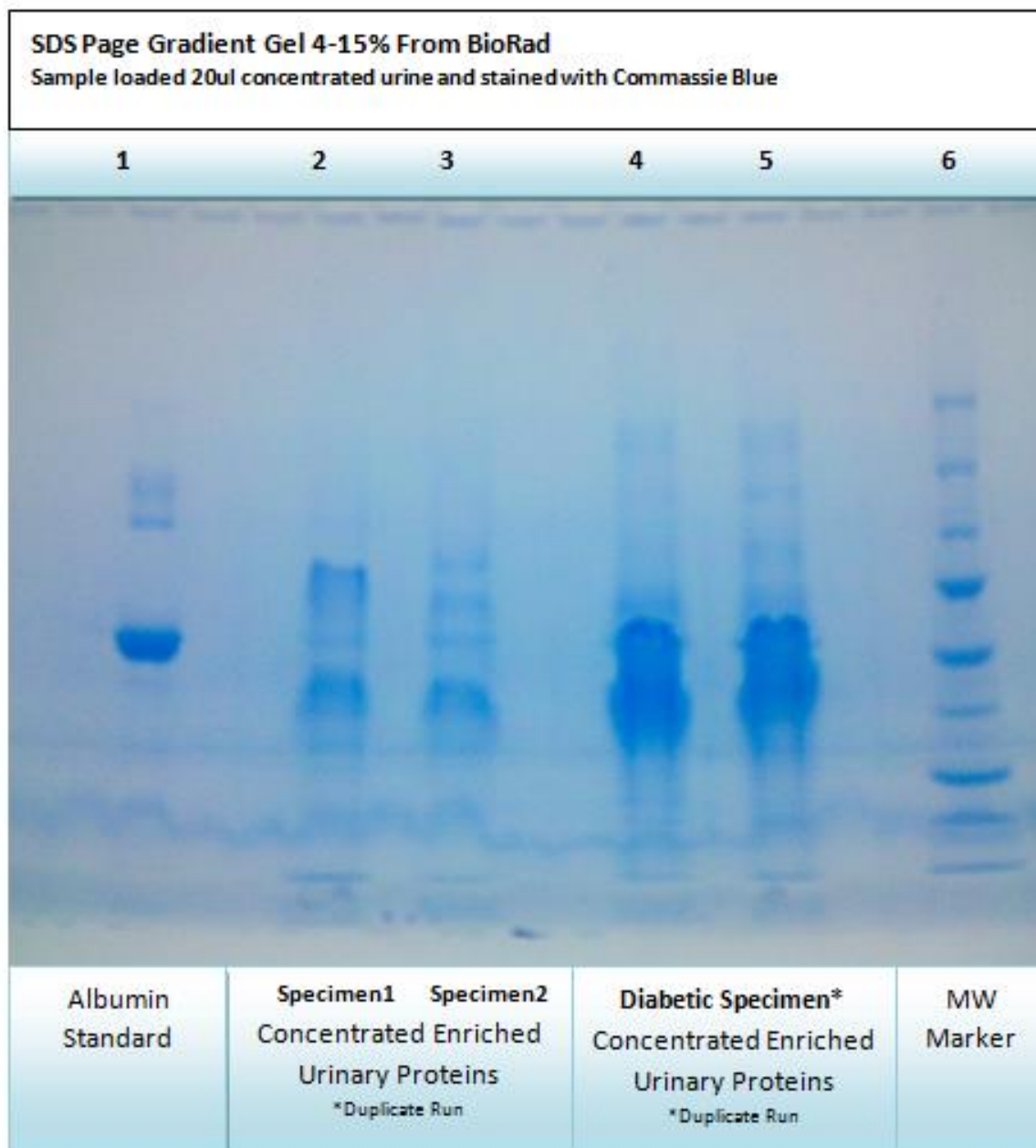
### *Urine Protein Enrichment For Urine Proteomics & Biomarkers*

- <60 minute bind, wash and elute protocol
- Linearly scalable up or down.
- Mild elution maintains tertiary structure and simple transfer to secondary analysis
- Applicable to 1 & 2 DE, proteomics, mass spec, and microarrays
- The eluted fractions retain their enzymatic and biological activity

UPCK™ Urine Protein Concentration Kit is a polymeric silica-based protein enrichment matrix designed as an alternative to ultra filtration and solvent precipitation. UPCK™ Urine Protein Concentration Kit has been especially optimized for proteomic studies of urine proteins.



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Product	Size	# of Samples & Sample Size*	Item No.	Price
<b>UPCK™ Urine Protein Concentration Kit</b>	10 Preps	10 preps, 10 ml urine samples per prep	UPCK-10	\$345
<b>UPCK™ Urine Protein Concentration Kit</b>	25 Preps	25 preps, 10 ml urine samples per prep	UPCK-25	\$750

Items Required	10 Prep	25 Prep	Reagent
UPCK™ matrix	0.75 grams	1.9 grams	<b>Supplied</b>
Binding Buffer UPBB, PH 6.0	60 ml	150 ml	<b>Supplied</b>
Wash Buffer UPWB, PH 7.0	10 ml	25 ml	<b>Supplied</b>
Elution Buffer UPEB, PH 10.0	10 ml	25 ml	<b>Supplied</b>
SpinX Centrifuge tube filters	10	25	<b>Supplied</b>
Conical centrifuge tube 50ml	10	25	<b>Not Supplied</b>
Wide Bore Pipette	-	-	<b>Not Supplied</b>

### PROTOCOL – Designed to concentrate or enrich protein from 10 ml of urine

- To 10 ml urine, add 5 ml UPCK™ binding buffer (UPBB™) in 50 ml conical centrifuge tube.
- Add 75 mg UPCK™ Urine Protein Concentration matrix and vortex for 25 minutes. Caution: Vortex adequately so that the resin does not settle at the bottom of the centrifuge tube.
- Allow the samples to settle for 10 minutes. Decant or pipette off the supernatant.
- Pipette UPCK™ protein bound matrix from Step 3 to the supplied Spin-X tube. Note: if all matrix does not transfer, use additional UPCK™ binding buffer (UPBB™ approximately 400ul) to resuspend the matrix & transfer again. Use wide bore pipette.
- Mix for 5 minutes and centrifuge at 10,000 rpm for 4 minutes. Discard the filtrate.
- Add 400 µl of UPCK™ Wash Buffer (UPWB™) to the pellet. Mix for 5 minutes and centrifuge at 10000 rpm for 4 minutes. Discard the filtrate.

**The bead is now enriched with urine proteins. For LC-MS sample preparation, an on-bead digestion protocol can be applied (protocol follows on next page). Otherwise proceed to the next step.**

- Add 400 µl of UPCK™ Elution Buffer (UPEB™) to the pellet. Vortex for 10 minutes and centrifuge at 10,000 rpm for 4 minutes. The enriched proteins is in the filtrate which is now ready for further analysis for example: LC-MS, LC-MS/MS, 1 & 2 DE, proteomics, mass spec, and microarrays, enzyme assays.
- This protocol can be scaled up or down



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### Suggested On-Bead Digestion Protocol

- ❖ After the final wash step from step 6, add 200 µls of 10 mM DTT solution to the beads for complete immersion, mix and incubate at 60°C for ½ hour.
- ❖ After cooling, add 200 µls of 50 mM iodoacetamide to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
- ❖ Centrifuge at 5000xg (medium setting, not max) for 3 mins, and discard supernatant.
- ❖ On-bead digestion is done by adding 300 µls of a 0.125 µg/µL (or calculated to a user preferred ratio – typically 50-100:1 w:w, protein:trypsin) of MS-grade Trypsin to the beads. Digest overnight at 37°C.
- ❖ Centrifuge at 5000xg (medium setting, not max) for 3 mins, and retain peptide filtrate.
- ❖ To further extract remaining peptides, add 300 µls of 10% solution of formic acid to the beads.
- ❖ Incubate for 15 minutes at 37°C, centrifuge at 5000xg (medium setting, not max) for 3 mins, and add this volume to the first volume.
- ❖ Reduce to final volume using a SpeedVac.

### Related Products

#### UPCK Buffers

UPCK Buffer Kit™

UPCK Wash Buffer UPWB™

UPCK Binding Buffer UPBB™

UPCK Elution Buffer UPEB™

### CONTACT US

**We welcome your questions and comments regarding our products.**

**Tel: 0032 16 58 90 45**

**Email: [info@gentaur.com](mailto:info@gentaur.com)**



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