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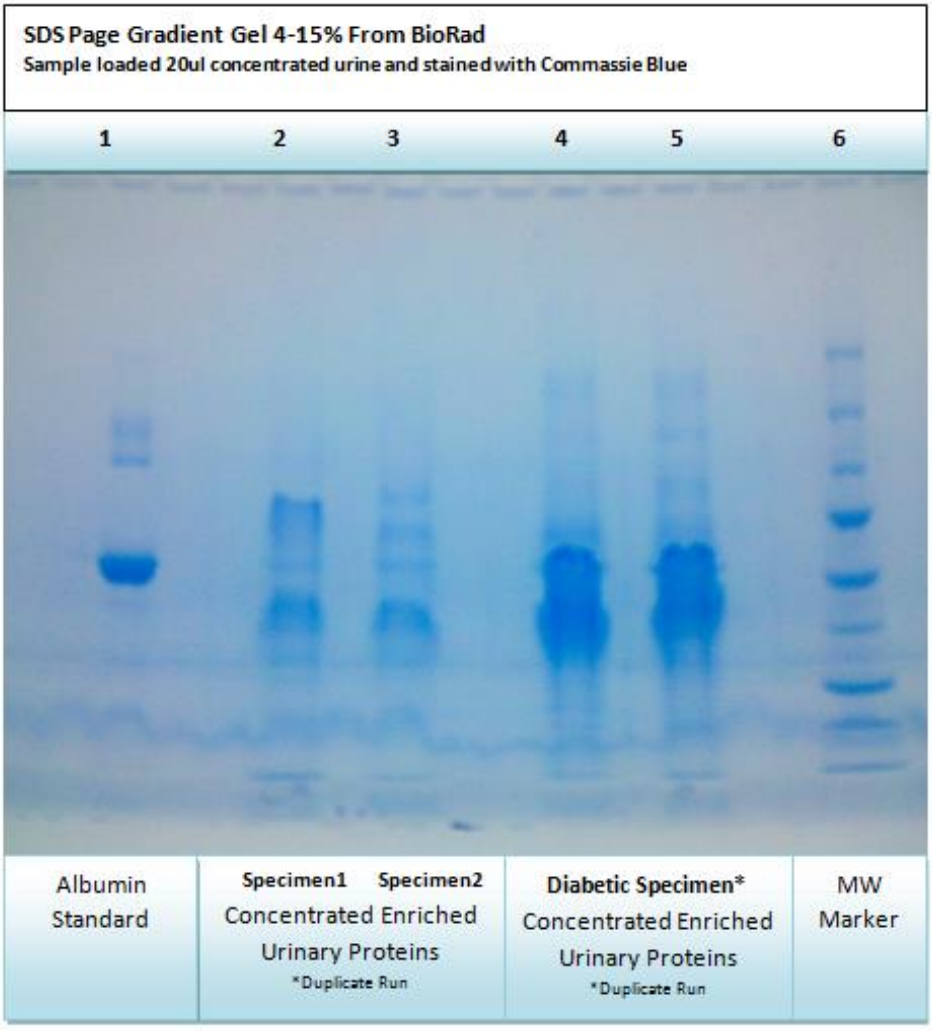
UPCK™ Urine Protein Concentration Kit

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
UPCK™ Urine Protein Concentration Kit	10 Preps	10 preps, 10 ml urine samples per prep	UPCK-10	$345
UPCK™ Urine Protein Concentration Kit	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	**Supplied**
Binding Buffer UPBB, *PH 6.0*	60 ml	150 ml	**Supplied**
Wash Buffer UPWB, *PH 7.0*	10 ml	25 ml	**Supplied**
Elution Buffer UPEB, *PH 10.0*	10 ml	25 ml	**Supplied**
SpinX Centrifuge tube filters	10	25	**Supplied**
Conical centrifuge tube 50ml	10	25	**Not Supplied**
Wide Bore Pipette	-	-	**Not Supplied**

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

1. To 10 ml urine, add 5 ml UPCK™ binding buffer (UPBB™) in 50 ml conical centrifuge tube.
2. 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.
3. Allow the samples to settle for 10 minutes. Decant or pipette off the supernatant.
4. 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.
5. Mix for 5 minutes and centrifuge at 10,000 rpm for 4 minutes. Discard the filtrate.
6. Add 400 µl of UPCK™ Wash Buffer (UPWB™) to the pellet. **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.**
7. Mix for 5 minutes and centrifuge at 10000 rpm for 4 minutes. Discard the filtrate.
8. 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.
9. This protocol can be scaled up or down

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Suggested On-Bead Digestion Protocol

- After the final wash steps from step 7, 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 ug/uL (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.

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