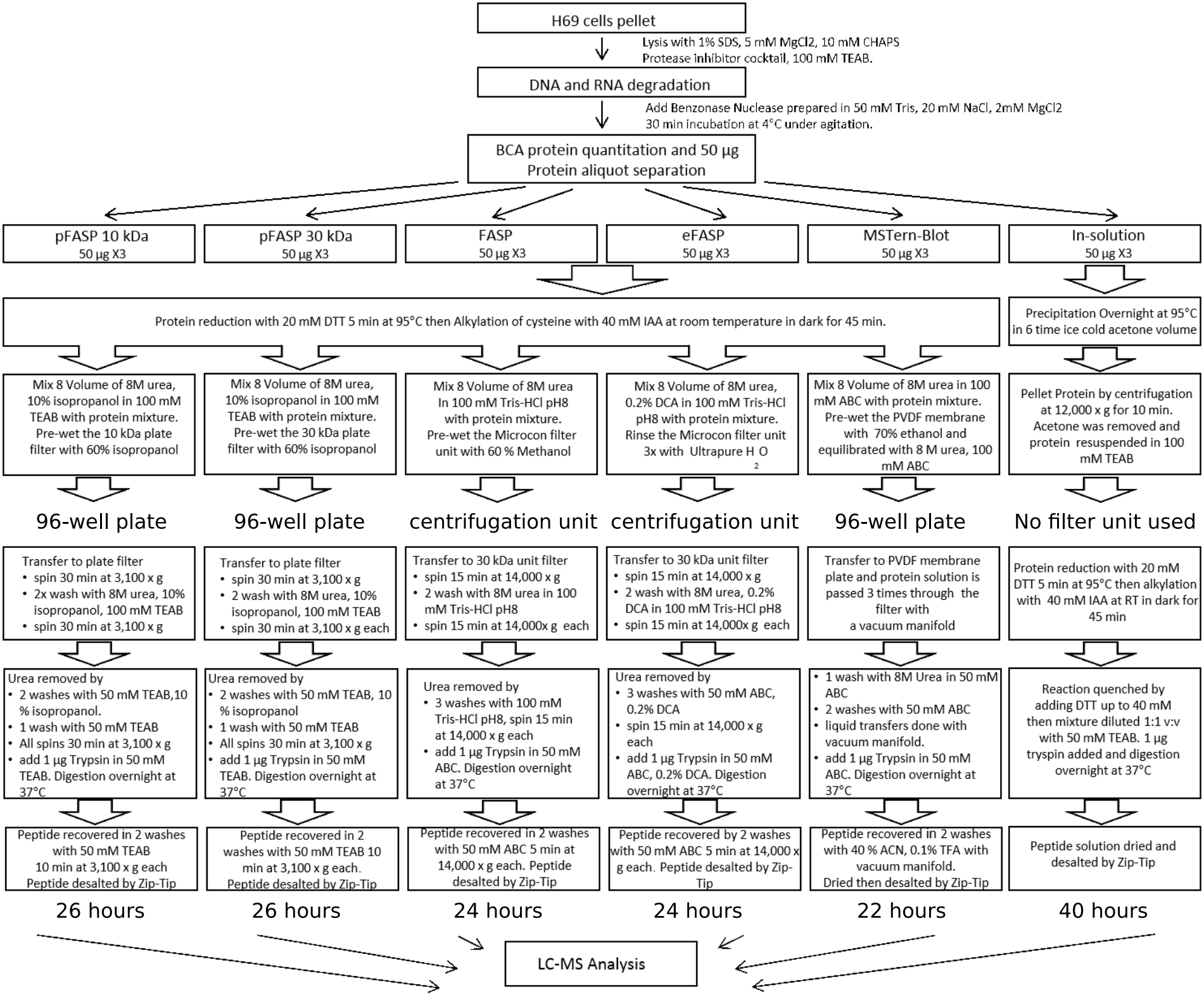
H69 cells pellet
Lysis with 1% SDS, 5 mM MgCl2, 10 mM CHAPS
Protease inhibitor cocktail, 100 mM TEAB.
DNA and RNA degradation
Add Benzonase Nuclease prepared in 50 mM Tris, 20 mM NaCl, 2mM MgCl2
30 min incubation at 4°C under agitation.
BCA protein quantitation and 50 µg
Protein aliquot separation
pFASP 10 kDa
50 µg X3
pFASP 30 kDa
50 µg X3
FASP
50 µg X3
eFASP
50 µg X3
MSTern-Blot
50 µg X3
In-solution
50 µg X3
Protein reduction with 20 mM DTT 5 min at 95°C then Alkylation of cysteine with 40 mM IAA at room temperature in dark for 45 min.
Precipitation Overnight at 95°C in 6 time ice cold acetone volume
Mix 8 Volume of 8M urea, 10% isopropanol in 100 mM TEAB with protein mixture. Pre-wet the 10 kDa plate filter with 60% isopropanol
Mix 8 Volume of 8M urea, 10% isopropanol in 100 mM TEAB with protein mixture. Pre-wet the 30 kDa plate filter with 60% isopropanol
Mix 8 Volume of 8M urea In 100 mM Tris-HCl pH8 with protein mixture. Pre-wet the Microcon filter unit with 60 % Methanol
Mix 8 Volume of 8M urea, 0.2% DCA in 100 mM Tris-HCl pH8 with protein mixture. Rinse the Microcon filter unit 3x with Ultrapure H O 2
Mix 8 Volume of 8M urea in 100 mM ABC with protein mixture. Pre-wet the PVDF membrane with 70% ethanol and equilibrated with 8 M urea, 100 mM ABC
Pellet Protein by centrifugation at 12,000 x g for 10 min. Acetone was removed and protein resuspended in 100 mM TEAB
96-well plate
96-well plate
centrifugation unit
centrifugation unit
96-well plate
No filter unit used
Transfer to plate filter
• spin 30 min at 3,100 x g
• 2x wash with 8M urea, 10% isopropanol, 100 mM TEAB
• spin 30 min at 3,100 x g
Transfer to plate filter
• spin 30 min at 3,100 x g
• 2 wash with 8M urea, 10% isopropanol, 100 mM TEAB
• spin 30 min at 3,100 x g each
Transfer to 30 kDa unit filter
• spin 15 min at 14,000 x g
• 2 wash with 8M urea in 100 mM Tris-HCl pH8
• spin 15 min at 14,000x g each
Transfer to 30 kDa unit filter
• spin 15 min at 14,000 x g
• 2 wash with 8M urea, 0.2% DCA in 100 mM Tris-HCl pH8
• spin 15 min at 14,000x g each
Transfer to PVDF membrane plate and protein solution is passed 3 times through the filter with a vacuum manifold
Protein reduction with 20 mM DTT 5 min at 95°C then alkylation with 40 mM IAA at RT in dark for 45 min
Urea removed by
• 2 washes with 50 mM TEAB,10 % isopropanol.
• 1 wash with 50 mM TEAB
• All spins 30 min at 3,100 x g
• add 1 µg Trypsin in 50 mM TEAB. Digestion overnight at 37°C
Urea removed by
• 2 washes with 50 mM TEAB, 10 % isopropanol
• 1 wash with 50 mM TEAB
• All spins 30 min at 3,100 x g
• add 1 µg Trypsin in 50 mM TEAB. Digestion overnight at 37°C
Urea removed by
• 3 washes with 100 mM Tris-HCl pH8, spin 15 min at 14,000 x g each
• add 1 µg Trypsin in 50 mM ABC. Digestion overnight at 37°C
Urea removed by
• 3 washes with 50 mM ABC, 0.2% DCA
• spin 15 min at 14,000 x g each
• add 1 µg Trypsin in 50 mM ABC, 0.2% DCA. Digestion overnight at 37°C
• 1 wash with 8M Urea in 50 mM ABC
• 2 washes with 50 mM ABC
• liquid transfers done with vacuum manifold.
• add 1 µg Trypsin in 50 mM ABC. Digestion overnight at 37°C
Reaction quenched by adding DTT up to 40 mM then mixture diluted 1:1 v:v with 50 mM TEAB. 1 µg tryspin added and digestion overnight at 37°C
Peptide recovered in 2 washes with 50 mM TEAB 10 min at 3,100 x g each Peptide desalted by Zip-Tip
Peptide recovered in 2 washes with 50 mM TEAB 10 min at 3,100 x g each. Peptide desalted by Zip-Tip
Peptide recovered in 2 washes with 50 mM ABC 5 min at 14,000 x g each. Peptide desalted by Zip-Tip
Peptide recovered by 2 washes with 50 mM ABC 5 min at 14,000 x g each. Peptide desalted by Zip-Tip
Peptide recovered in 2 washes with 40 % ACN, 0.1% TFA with vacuum manifold. Dried then desalted by Zip-Tip
Peptide solution dried and desalted by Zip-Tip
26 hours
26 hours
24 hours
24 hours
22 hours
40 hours
LC-MS Analysis