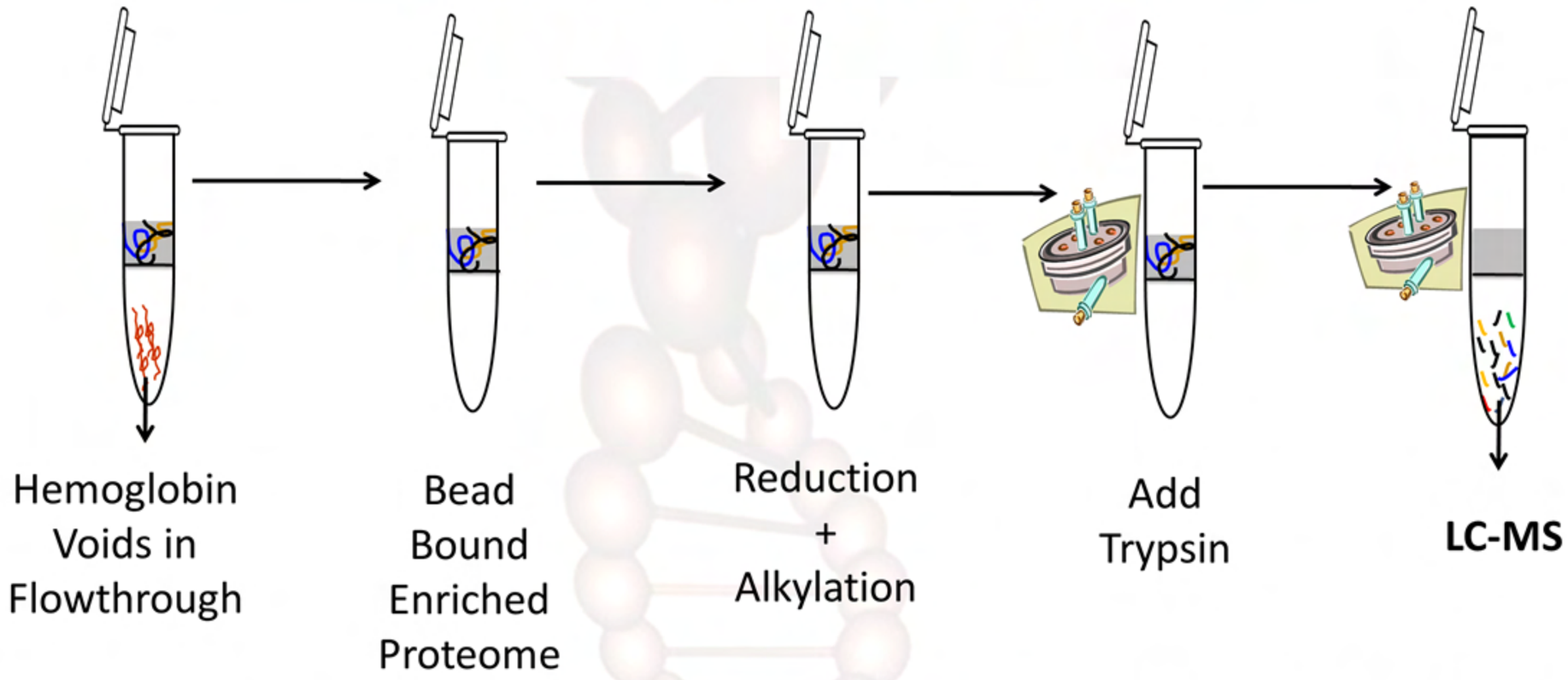
BIOTECH SUPPORT GROUP

HemoVoid™ On-Bead Digestion Application Data

Hemoglobin Depletion + Digestion Efficiency + Simple Workflows = Better LC-MS Output



Detailed Hemoglobin depletion protocol can be found on HemoVoid™ product sheet.

Digestion on HemoVoid™ Matrix

Courtesy of Irene Granlund, Umeå University, Umeå, Sweden

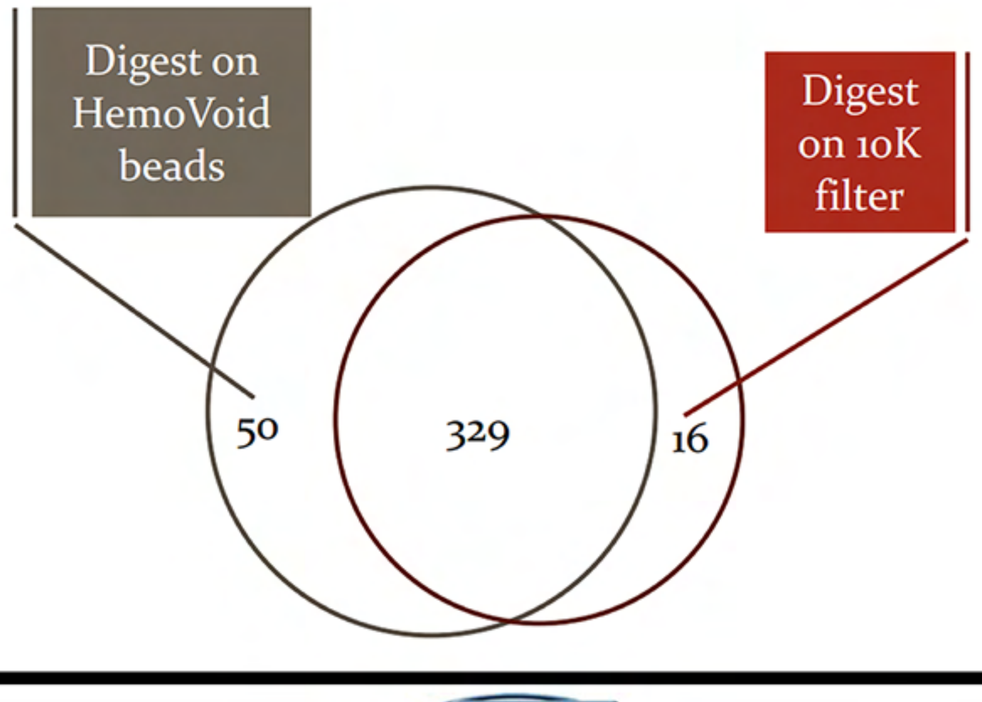
Digestion on Spin-X® tube

1. After the final wash steps from Protocol provided for HemoVoid™, add 100 µL of 5 mM DTT in HVWB to the beads for complete immersion, mix and incubate at 60°C for 1 hour.
2. Add 100 µL of 25 mM Iodoacetamide in HVWB (end concentration 12.5 mM) to the DTT/bead suspension, mix and incubate in the dark, 37°C for ½ hour.
3. Centrifuge at 3,200 x g for 2 minutes, and discard supernatant. Wash the beads with 200 µL HVWB, mix and centrifuge at 3,200 x g for 2 minutes.
4. Move the HemoVoid™ device with Hemoglobulin depleted proteins to new clean tubes.
5. Add 100 µL of digestion solution (5ng/µL trypsin in 50 mM NH_4HCO_3-solution) to the beads. Incubate 37°C, overnight (15 hours).
6. Centrifuge down at 3,200 x g for 2 minutes.
7. Wash HemoVoid™ matrix 2 times with 150 µL 50 mM NH_4HCO_3, mix and centrifuge down, at 3,200 x g for 2 minutes.

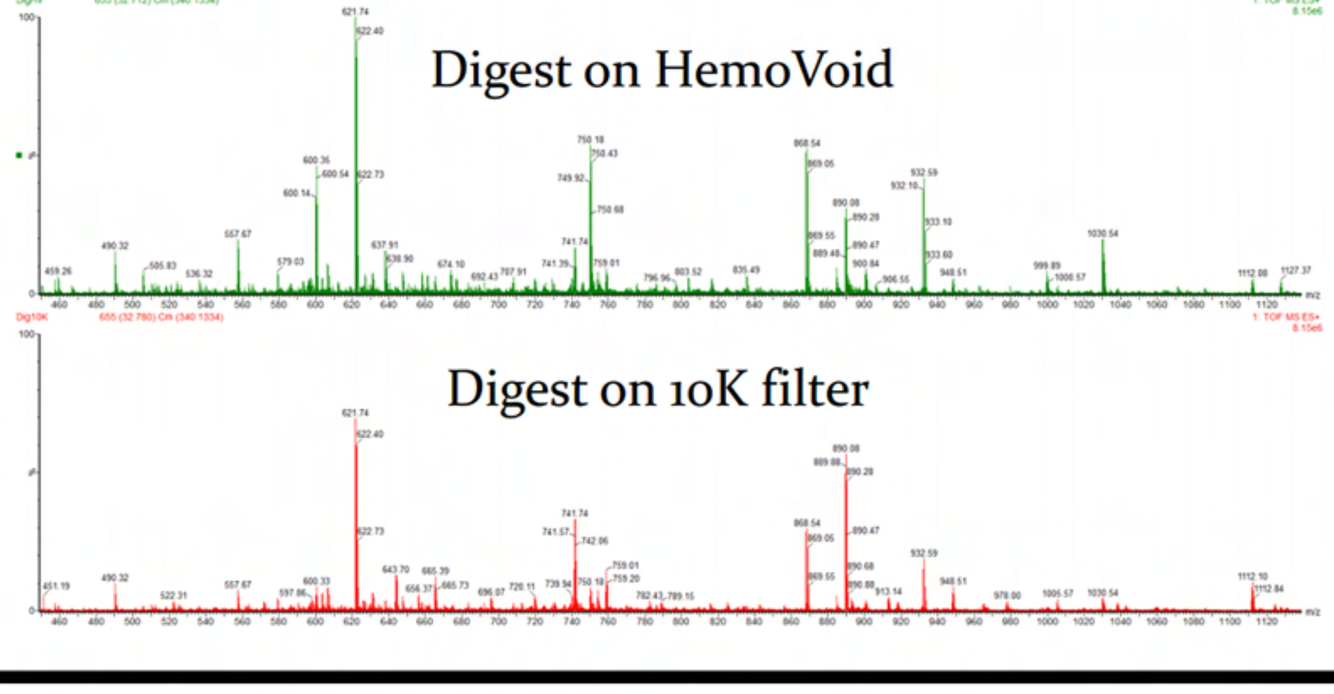
Digestion on 10K filter device

1. The proteins are eluted according to the Protocol provided with 300 µL Elution Buffer HVEB. Mixed for 10 minutes and centrifuged 4 min. at 9,000 x g.
2. The eluted sample is transferred to 10K filter device, (NanoSep 10K Omega, centrifugal device, Pall Life Sciences). The samples are centrifuged down on the filter (the solution under filter is thrown away).
3. Add 300 µL 19.5 mM DTT in Guanidine solution (6 M guanidine, 0.1 M Tris, 5 mM EDTA, pH ~8). Incubate at 60°C for 1 hour.
4. Add 100 µL 81 mM Iodoacetamide in Guanidine solution (end concentration 20 mM). Alkylate in the dark, 37°C for ½ hour.
5. Spin down 14000 x g to remove liquid.
6. Wash filter two times with 200 µL 50 mM NH_4HCO_3, spin down at 14 000 x g in between.
7. Move the 10K filter to new clean tubes.
8. Add 100 µL of digestion solution (5ng/µL trypsin in 50 mM NH_4HCO_3-solution) on the filter. Incubate 37°C, overnight (15 hours).
9. Wash filter with 2 times with 150 µL 50 mM NH_4HCO_3. Spin down at 14 000 x g.

IDENTIFIED PROTEINS



SPECTRUM



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