

## SDS-PAGE of Captured Proteins and LC-MS Analysis

**NOTE: Before proceeding this protocol, please read carefully the instruction in the handbook “Guideline for CCMS”! This is only a brief description of the procedure!**

### SDS-PAGE

Add 10 µl 2x Laemmli sample buffer to the Streptavidin-magnetic-beads-protein-complexes collected after the last wash step, homogenize, incubate 10 min at 95 °C and load the gel with the whole suspension. Alternatively, the beads may be separated by using the caproMag and only the supernatant may be loaded on the gel.



**Note:** When the whole suspension was loaded wash the slots after the run with water before proceeding fixation the gels.

Recommended downstream analysis:

SDS-Page: Pre-cast gels from OLS (1 mm mini gels, 4-20 %, 12 lanes). Run the gel at 120 V and 4 °C.

Silver staining: ProteoSilver™ Plus Silver Stain Kit from Sigma.

### In-gel Tryptic Digestion

Prepare all reagents freshly prior to use.

#### Excision of protein bands

- Wash the gel for 10 min with LC-MS-grade water, discard water and repeat washing step
- Cut as close as possible to the protein band to reduce the amount of "background" gel
- **Optional:** fragment the excised pieces into nearly 1 mm<sup>3</sup> cubes and transfer them to a clean siliconised 0.5 ml PCR tube.

#### Washing of gel pieces

- Wash the gel pieces each 15 min and discard supernatant after each step:
  - 100 µl water
  - 100 µl 50 % ethanol (v/v)
  - 100 µl water

- 100 µl 50 % ethanol (v/v)
- Add 50 µl 100 % ethanol and incubate for ~5 min to shrink the gel pieces (visual inspection), discard supernatant

#### Optional: Reduction and alkylation

- Re-swell the gel pieces in 50 µl 10 mM dithiothreitol (DTT)/100 mM  $\text{NH}_4\text{HCO}_3$  and incubate for 45 min at 56 °C
- Cool down tubes to room temperature
- Remove DTT solution
- Incubate gel pieces in 50 µl 55 mM iodoacetamide (IAA)/100 mM  $\text{NH}_4\text{HCO}_3$  for 30 min at room temperature in the dark
- Remove IAA solution

#### Washing of gel pieces

- Wash the gel pieces each 15 min and discard supernatant after each wash step:
  - 100 µl water
  - 100 µl 50 % ethanol (v/v)
  - 100 µl water
  - 100 µl 50 % ethanol (v/v)
- Add abs. ethanol (50 µl) and incubate 5 min to shrink the gel pieces (visual inspection), discard supernatant

#### Digestion

- Rehydrate gel pieces in a solution of 12.5 ng/µl of trypsin/50 mM  $\text{NH}_4\text{HCO}_3$  on ice, start with 10 µl, incubate for 20 min on ice
- Add more buffer if the initially added volume has been absorbed by the gel pieces and further incubate up to 45 min incubation on ice
- Replace supernatant by 5-20 µl (depending on size of the gel pieces) of 50 mM  $\text{NH}_4\text{HCO}_3$  without trypsin
- Close the lids of the tubes well, wrap the whole rack with the tubes in parafilm and incubate over night at 37 °C

### Extraction of peptides

- Transfer supernatant with peptides to a clean 0.5 ml siliconised tube
- Add enough of 5 % formic acid to cover the gel pieces (~ 20 µl), do **NOT** discard supernatant
- Add the same volume of acetonitrile and incubate for 15 min
- Remove supernatant into the clean 0.5 ml siliconised tube above
- Add enough of 5 % formic acid to cover the gel pieces (~ 20 µl), do **NOT** discard supernatant
- Add the same volume of acetonitrile and incubate for 15 min
- Remove supernatant into the clean 0.5 ml siliconised tube above
- Dry the sample in a vacuum centrifuge

### Optional: Desalting

- Re-dissolve the peptides in 5-10 µl of 5 % formic acid, sonicate briefly
- For desalting we recommend Stage tips® available from Proxeon Biosystems ([www.proxeon.com](http://www.proxeon.com))
- Follow the manufacturer's instruction

### Mass spectrometry

- Re-dissolve the peptides in 5-10 µl of 0.1 % formic acid, sonicate briefly
- **Optional:** Centrifuge for 10 min at 12.000 x g
- Inject peptide extract into MS

### *"OnBead" Digestion*

#### Washing of proteins

- Wash the Streptavidin magnetic beads six times with 200 µl 80 % acetonitrile  
**Note:** During the first washing steps the bead suspension could agglutinate, resuspend well by using a pipette
- Wash the Streptavidin magnetic beads one time with 200 µl LC-MS-grade water, discard supernatant

#### Optional: Reduction and alkylation

- Add 20  $\mu$ l of 10 mM DTT/100 mM  $\text{NH}_4\text{HCO}_3$ , mix the sample by gently vortexing and incubate for 1 h at 56 °C
- Remove DTT solution
- Add 20  $\mu$ l of 55 mM IAA/100 mM  $\text{NH}_4\text{HCO}_3$ , mix the sample and incubate for 30 min at room temperature in the dark
- Remove IAA solution

#### Digestion

- Add 10  $\mu$ l of 50 mM  $\text{NH}_4\text{HCO}_3$ /0.5 mM  $\text{CaCl}_2$ , 200 ng/ $\mu$ l trypsin,
- Close the lids of the tubes well, wrap the whole rack with the tubes in parafilm and incubate over night at 37 °C on a temperature controlled shaker

#### Optional: Desalting

- Re-dissolve the peptides in 5-10  $\mu$ l of 5 % formic acid, sonicate briefly
- For desalting we recommend Stage tips® available from Proxeon Biosystems ([www.proxeon.com](http://www.proxeon.com))
- Follow the manufacturer's instruction

#### Mass spectrometry

- Re-dissolve the peptides in 5-10  $\mu$ l of 0.1 % formic acid, sonicate briefly
- **Optional:** Centrifuge for 10 min at 12.000 x g
- Inject peptide extract into MS

## Contact

### Headquarters

**caprotec bioanalytics GmbH**  
Volmerstrasse 5  
D-12489 Berlin

Phone: +49 30 63 92 39 90

Fax: +49 30 63 92 39 89

Web: [www.caprotec.com](http://www.caprotec.com)

Email: [sales@caprotec.com](mailto:sales@caprotec.com)

**caprotec Inc., USA**

15 New England Executive Office Park  
Burlington, MA 01803, USA

Phone: + 1 781 685 4992

Fax: +1 781 685 4601

Web: [www.caprotec.com](http://www.caprotec.com)

E-Mail: [info@caprotec.com](mailto:info@caprotec.com)

### Copyright

© 2008 caprotec bioanalytics GmbH. All rights reserved. Reproduction in whole or in part only with permission of caprotec bioanalytics GmbH.

### Trademarks

caprotec, caproKit, Capture Compound, caproBeads, caproBox, caproMag and ImproMed are registered trademarks of caprotec bioanalytics GmbH. All other used tradenames or trademarks belong to their respective proprietaries.

**FOR RESEARCH ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**

### Products & Services

CCMS technology is made available as ready to use caproKit reagents and services.

For more information please visit [www.caprotec.com](http://www.caprotec.com)

Or contact us. Email: [info@caprotec.com](mailto:info@caprotec.com)

Phone: +49 30 6392 4004

Products and Services are for Research use only.

© 2008 caprotec bioanalytics GmbH