TECHNICAL NOTES SERVA

Biochemicals

Electrophoresis Bioseparation

Life Sciences

- Specials
- **ICPL[™]** Quadruplex Kit: Workflow Comparative analysis of four different cell types or cell phases A D Æ 4cell type/ cell type/ cell type/ cell type/ cell phase 2 cell phase 3 cell phase 4 cell phase 1 **Denaturation and alkylation** ICPL[™] labelling **Combination of four samples** Reduction of the complexity on protein level (by 1DGE, 2DGE, LC, FFE...) no 1D 2D White LC FFE separation Enzymatic digestion of proteins in peptides 10 Reduction of the complexity on peptide level Mass spectrometry (quantification, identification)
 - Comperative analysis of up to four proteomes
 - Four different labels for ICPL technology available
 - Analysis of posttranslational modifications and isoforms

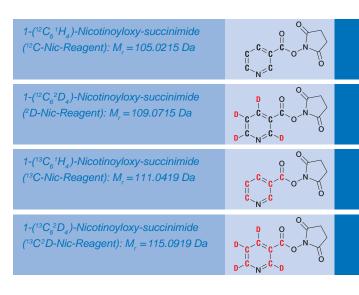
SERVA ICPL[™] Quadruplex Kit

Quantitative MS analysis of proteomes

ICPL[™]-4-SUCCESS

The powerful ICPL-technology for comparative quantification of proteins is now available with a fourth label (see below). Applying the ICPL quadruplex method the simultaneous quantitative analysis of four independent proteome samples can be performed by stable protein labelling side by side. The ICPL-technology combines the power of Isotope Coded Protein Labelling (ICPL) with an unmatched dynamic range for protein identification and quantification due to its potential combination with intact protein fractionation steps (see workflow).

Introducing the ICPL quadruplex method at HUPO 2008 in Amsterdam, The Netherlands (Halder, Thomas M. et al., ICPL (Isotope-coded Protein Labelling) QUADRUPLEX Technology for quantitative Proteomics) a new software suite called ICPL *Quant* also was developped to analyse ICPL labelled LC/MS data. The main functionalities are peptide pattern searching (duplex, triplex or quadruplex), peptide quantification in an LC-run and merging with MASCOT protein results. ICPL *Quant* takes deisotoped data as input, generates precursor files for MS/MS and is able to import MASCOT result files for combining MS/MS and quantification results.



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Electrophoresis

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Quantitative MS analysis of proteomes

Comparative analysis of proteomes

The new experimental approach (details see "Workflow") enables to compare the accurate and reproducible quantification of proteins. Protein fractionation provides a more sensitive and, therefore, a higher proteomes coverage. Protein labelling occurs prior to the chromatographic/electrophoretic separation thus maintaining the quantitative ratios. Even proteins, which are superimposed in the 2D-gel, as well as isoforms, posttranslational modified proteins and proteolysis products can be identified and yet more important, quantified. The ICPL Quadruplex Kit is designed for use with protein fractionation such as 1D/2D PAGE and post digestion LC-MS/MS analysis.

The quantitative analysis of up to four highly complex protein mixtures can be significantly improved in comparison to until now available "Stable Isotope Label"methods by the development of the ICPLQuadruplex Kit (ICPL = Isotope Coded Protein Labelling). The labelling on protein level has the following advantages:

- a) In contrast to other labelling techniques the ICPL Quadruplex Kit permits proteome analysis on 2D-gel-basis as well as on multidimensional LC-MS-basis using ESI-MS or MALDI-MS.
- b) All the approaches that work on a truly proteomics scale use protein pre-fractionation such as LC, PAGE, 2D-GE or magnetic beads. Protein fractionation is compatible with the ICPL-technology, as quantitative ratios remain constant throughout any fractionation steps. This reduces complexity in each protein fraction which increases the dynamic range of protein detection and quantification and thus the number of identified proteins.
- c) A high sequence coverage of labelled peptides provides a sound statistical base for automatic protein quantification avoiding excessive manual validation efforts.
- d) Modified peptides are detected by MS at increased sensitivity.

Mass difference between modified amino groups of proteins labelled with:

| Reagent | ¹² C-Nic | ² D-Nic | ¹³ C-Nic | ¹³ C ² D-Nic |
|------------------------------------|---------------------|--------------------|---------------------|------------------------------------|
| ¹² C-Nic | 0 Da | 4.05 Da | 6.0204 Da | 10.0704 Da |
| ² D-Nic | 4.05 Da | 0 Da | 1.9704 Da | 6.0204 Da |
| ¹³ C-Nic | 6.0204 Da | 1.9704 Da | 0 Da | 4.05 Da |
| ¹³ C ² D-Nic | 10.0704 Da | 6.0204 Da | 4.05 Da | 0 Da |

SERVA´s partner for the ICPL[™]-technology



TOPLAB has provided an exclusive distribution license for the ICPL-technology to SERVA. TOPLAB as a service company carries out complete proteome analysis by customer order, but supports as well customers with regard to all technical questions concerning ICPL (www.toplab.de).

SERVA's partner in marketing of the ICPL[™]-technology worldwide

BRUKER DALTONICS[®]

The SERVA ICPL Quadruplex Kit can also be obtained from Bruker Daltonics (Cat. No. BDAL #244574). For more information please contact Bruker Daltonics directly.

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www.bdal.de/care

Ordering Information

| Product | Cat. No. | Size |
|--|----------|-----------------|
| SERVA ICPL [™] Quadruplex Kit | 39232.01 | 4 x 6 Reactions |
| SERVA ICPL™ Kit | 39230.01 | 2 x 6 Reactions |





