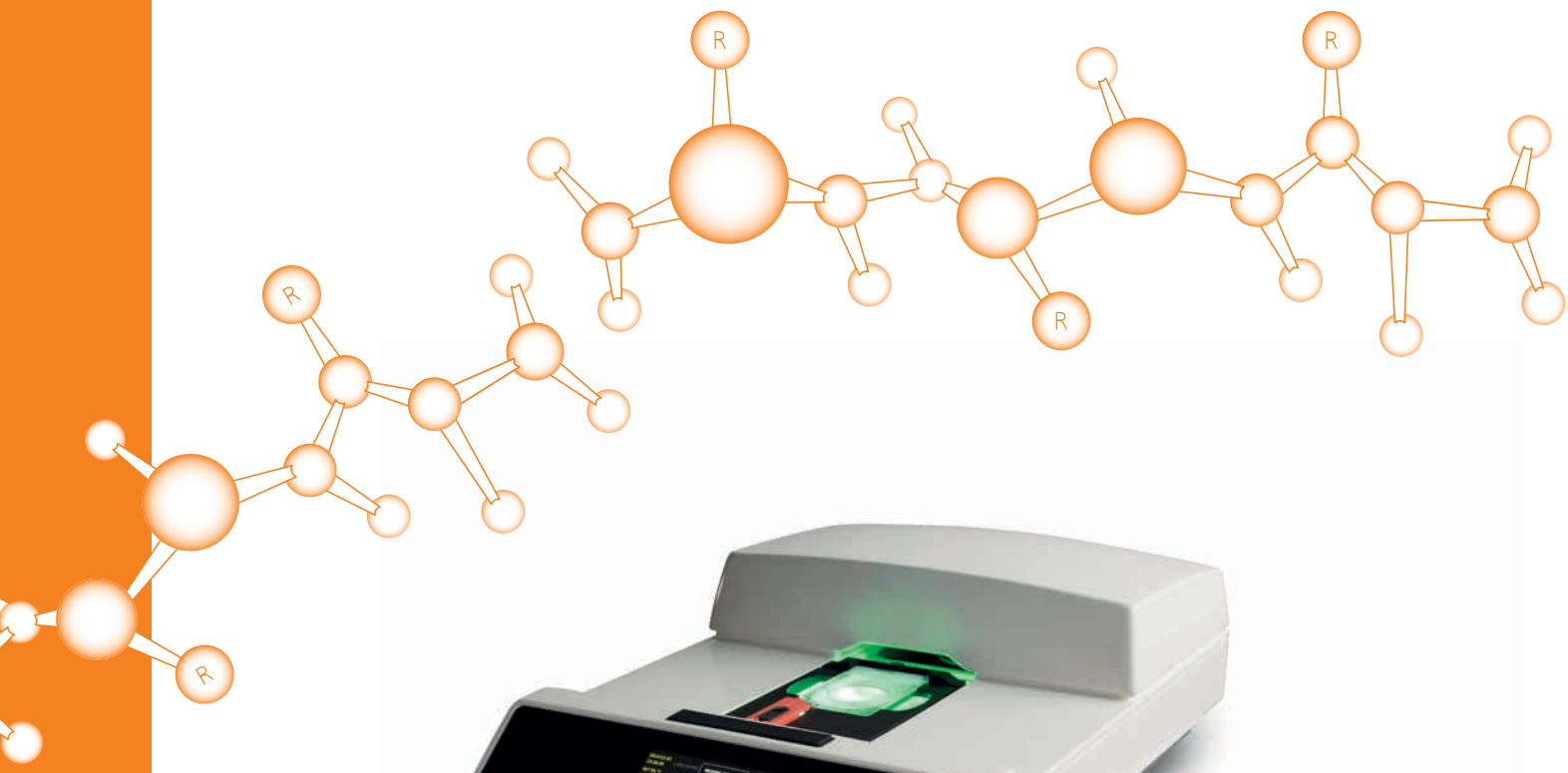
# Maximize analytical success using state-of-the-art sample preservation







denator
STABILIZING THE PROTEOME

# Meeting the challenge of biological change post-sampling

The act of biosampling introduces rapid, tissue-specific changes in the cellular environment that begin immediately after excision. Cellular signaling remains active or rather reactive and proteins begin to degrade, causing significant changes to the proteome. This can lead to grave uncertainty in your analytical results. In order to maintain sample stability, preservation as early as possible after sampling is fundamental.

Heat stabilization is a revolutionary sample preservation technique that stops biological change immediately and permanently by inactivating enzymes. It is the perfect solution for anyone engaged in protein, peptide and biomarker research who wants their analytical result to reflect the *in vivo* state as closely as possible.

- When analyzing peptides, phosphorylations or small molecules
- Stops biological change immediately and permanently, and preserves the *in vivo* information
- Improves signal-to noise ratio, enabling detection of low abundant molecules

## Permanent, additive-free stabilization of fresh or frozen samples

The heat stabilization technology utilizes controlled conductive heating, to generate rapid homogenous thermal denaturation of biological samples. Conventional approaches such as inhibitor cocktails and pH changes are reversible while cross-linking utilizes toxic compounds. Any of these solutions may interfere with your downstream analytical techniques. Heat stabilization is permanent, additive-free, and can be used for fresh or frozen tissue.

*"The Stabilizor system is ideal for all tissue samples that you have stored in the freezer, as you really do preserve the protein profile from whatever state the tissue was in when you put it in the system - you preserve it "*

Prof. K. Gardiner, Department of Pediatrics, University of Colorado, USA

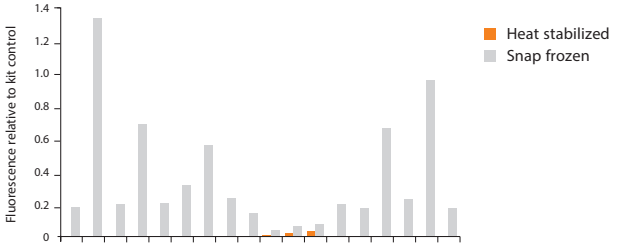
# Preserve phosphorylation states – compare true biological variation

Determining, as closely as possible, the *in vivo* status of phosphoproteins is crucial to understanding their role, yet phosphorylation states are highly sensitive to disturbances and can change rapidly. By using heat stabilization immediately after sampling, enzymatic activity is permanently stopped, allowing for comparison of true biological variation.

## Heat stabilization abolish 99.6% of kinase activity

*Fig 1.* 18 most phosphorylated peptides from a 144 peptide array in a kinase activity assay. Only three were measurable phosphorylated in heat stabilized samples.

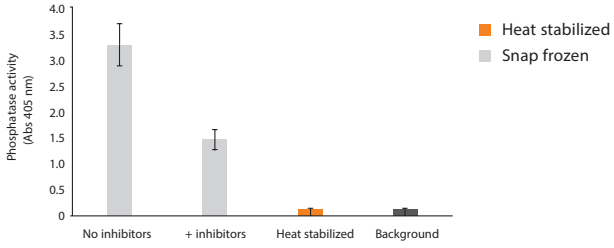
*Results adapted from; Thermal stabilization of tissues and the preservation of protein phosphorylation states for two-dimensional gel electrophoresis. Smejkal et al., Electrophoresis, 2011*




## Heat stabilization permanently inactivate phosphatases

*Fig 2.* Enzymatic activity in snap frozen mouse cortex samples compared to heat stabilized (n=5). Phosphatase activity was measured using colorimetric pNPP phosphatase kit (AnaSpec). Snap frozen samples were solubilized in buffers with and without inhibitors (PhosSTOP and c0mplete, Roche) using microtip sonication. The activity in the heat stabilized samples was equivalent to background levels.

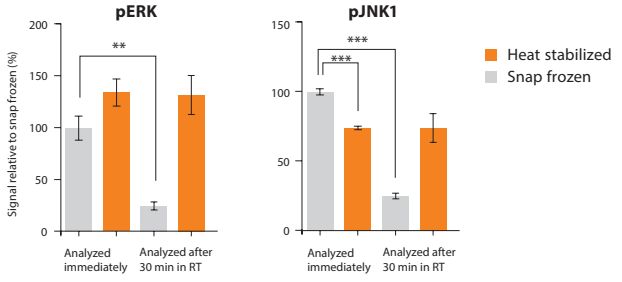
*Heat stabilization of the Tissue Proteome: A New Technology for Improved Proteomics. Svensson et al., J Proteome Res., 2009*




## Heat stabilization preserves actual phosphorylation states

*Fig 3.* Quantification of phosphoproteins in mouse hippocampal lystates. Heat stabilized and snap frozen samples with inhibitors, were analyzed by western blot immediately or after 30 min in room temperature (RT). (Student's t-test, **P<0.01, ***P<0.001)

*Preserving protein profiles in tissue samples: Differing outcomes with and without heat stabilization. Ahmed et al., Journal of Neuroscience Methods, 2011*




*"At last we have a solution to be able to preserve phosphorylated proteins in clinical samples"*

Prof. P. James, CREATE Health, Division of Protein Technology, Lund University, Sweden

# Detect more and novel endogenous peptides

Post-sampling proteolytic activity begins immediately, producing protein fragments that can mask endogenous peptides present at low levels. Rapid heat stabilization stops enzymatic changes and protein degradation immediately and permanently.

## Heat stabilization enables detection of low abundant endogenous peptides. Snap freezing reveals fewer peptides and shows signs of degradation.

*Fig 4.* LC-MS profile of heat stabilized bovine hypothalamus. Peaks (*) corresponding to endogenous neurosecretory peptides; VGF[489–506], CCK[21–44], GnRH, AL11, CLIP, HCNP and BAM-1745.

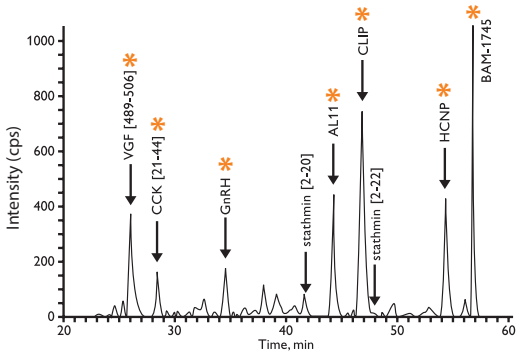



*Fig 5.* LC-MS profile of snap frozen bovine hypothalamus. The most prominent peaks (*) are degradation fragments of the Stathmin protein; Stathmin [2–20] and Stathmin [2–22]. Stathmin fragments are used as a marker of poor sample quality.

*Fig 4 and 5: Neuropeptide profiling of the bovine hypothalamus: Thermal stabilization is an effective tool in inhibiting post mortem degradation. Colgrave et al., Proteomics, 2011*

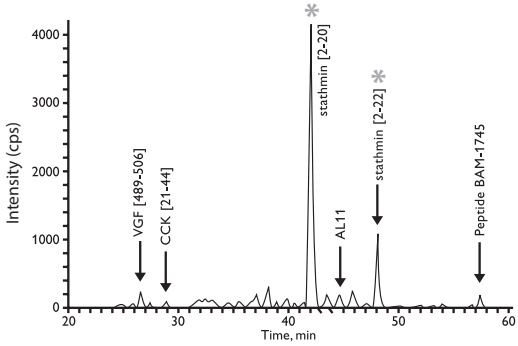
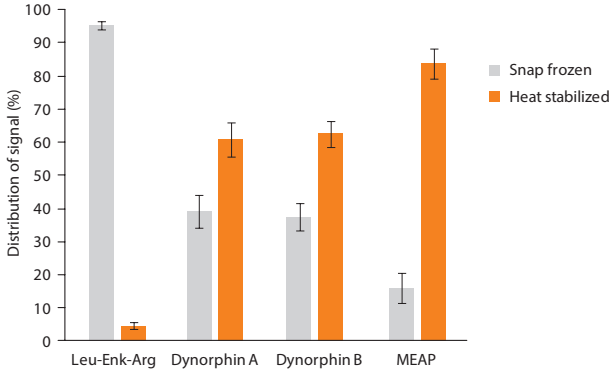



*Fig 6.* Dynorphin measured with RIA clearly show rapid degradation in the snap frozen sample, resulting in high levels of the metabolite Leu-Enkephalin-Arg. In heat stabilized samples the levels of parent peptides, Dynorphin A, Dynorphin B and MEAP remain high.

*Results courtesy of Professor Ingrid Nylander, (Uppsala University)*




*"We discovered several novel, potentially bioactive, brain neuropeptides"*

Prof. Per Andrén, Lab for Medical Mass Spectrometry, Uppsala University

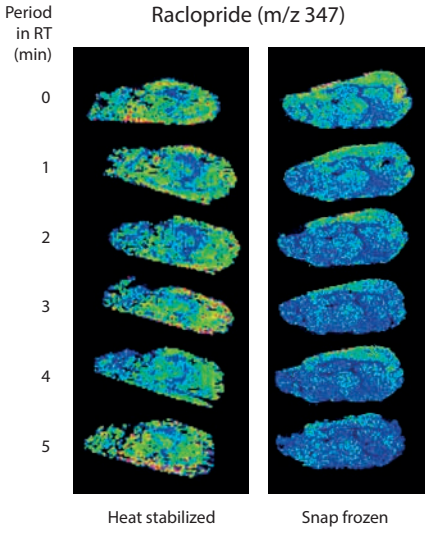
# Preserve small molecules in tissue samples

Metabolic activity continues post-sampling, causing variations to the molecular content of tissue sections. To maintain the sample quality, preservation as early as possible after tissue harvesting is fundamental.

## Heat stabilization enables detection of unstable small molecules with MALDI Mass Spectrometry Imaging

*Fig 7.* A comparison of heat stabilized samples and snap frozen mouse brains showed that the distribution of Raclopride remained constant in heat stabilized samples over time. In snap frozen samples, the amount of the molecule continuously decreased due to rapid degradation.

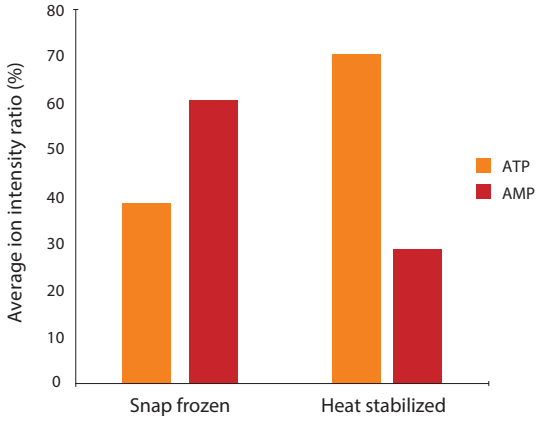
*Results courtesy of Dr. Richard Goodwin (AstraZeneca ) and Prof. Per E. Andrén (Uppsala University).*




## Heat stabilization permanently stops metabolic activity causing conversion of ATP to AMP

*Fig 8.* A comparison of the levels of ATP and AMP in both snap-frozen and heat stabilized mouse brain showed that ATP was detected at higher levels in heat stabilized samples compared to AMP, whereas the reverse situation was found in snap frozen samples.

*Results adapted from; Localisation of adenine nucleotides in heat-stabilised mouse brains using ion mobility enabled MALDI imaging. Blatherwick, E. Q., et al., International Journal of Mass Spectrometry, 2013.*

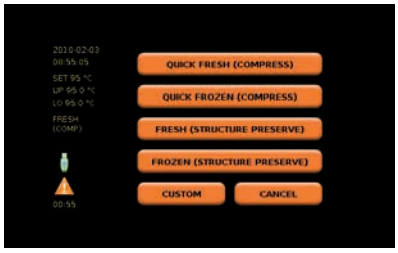



*"The heat stabilization technology by Denator was valuable to our extensive mapping project. When building a reference database we need to secure the best possible correlation with the in vivo situation, and this technology enabled us to ensure exactly that"*

Prof. Jesper Olsen, NFF Center for Protein Research, Denmark

# Simple steps to maximize analytical results

Heat stabilization is carried out using the Stabilizor™ system. The fully automated procedure ensures consistent and reproducible treatment of samples. Algorithms use details of the physical state (fresh or frozen) and sample size (measured automatically by lasers) to determine the exact amount of energy required for complete denaturation.




1. Select program for fresh or frozen samples, input sample details.

2. Start run-log to record time at the moment of sampling.

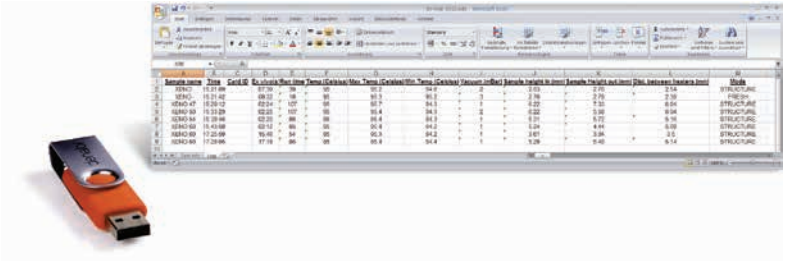
3. Place fresh or frozen sample in Maintainor® tissue card.

4. Place sealed Maintainor® tissue card into Stabilizor™ system.

5. Air is evacuated and sample enters instrument.

6. Green light signals completion of heat stabilization.

7. Remove and analyze directly or store at -80°C.



8. Link upstream processing with downstream results: export sample-specific parameters to Microsoft Excel *e.g.* sample ID, card ID, time after excision, before heating and heating time.

*" An important tool that will help to further our understanding of the molecular basis of biological processes in health and disease"*

Prof. Michael Dunn, UCD Conway, Ireland

# Heat stabilization and sample storage – the essentials

## Stabilizor™ T1 system.

Performs rapid controlled high temperature heating of biological samples.

## Maintainor® Tissue cards and storage box.

Heat stabilize, transport and store tissue samples. Inert materials minimize the risk of contaminants interfering with analysis.

## Ready-to-use sample preparation kits – for consistent analyses

Following an optimized and validated protocol saves time and helps to ensure consistent analytical results. Stabilizor™ Extraction Kits contain protocol, reagents and accessories required to prepare samples prior to 2D-GE or LC-MS. Final sample conditions may also be suitable for other analytical techniques. More details about the Stabilizor™ Extraction Kits are available at www.denator.com

## Stabilizor™ 2D-GE Extraction Kit

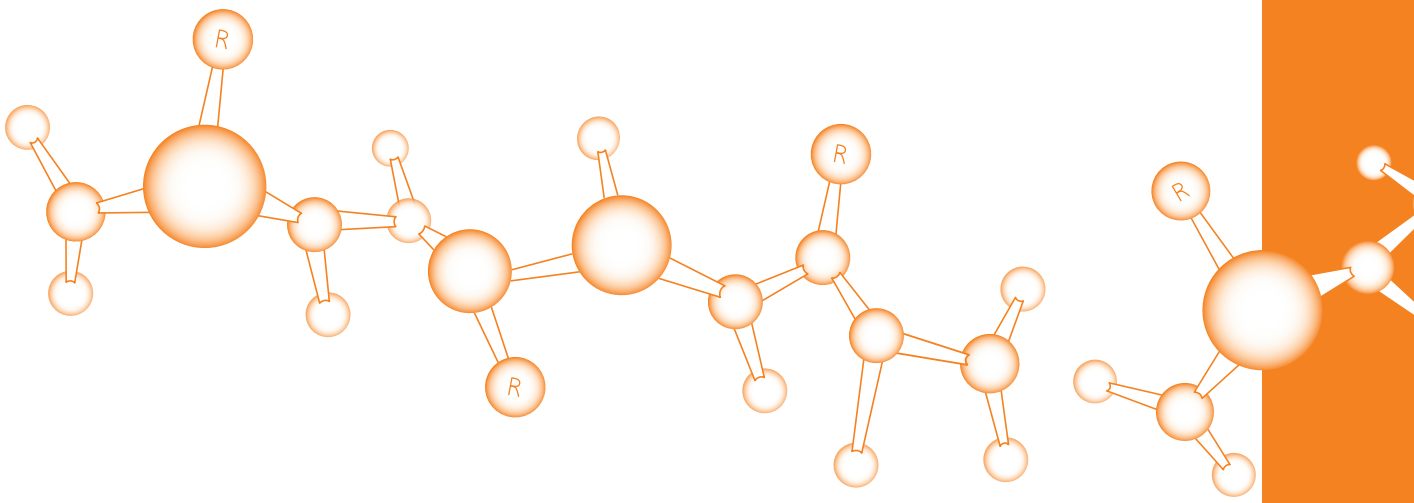
Maximize recovery of proteins. Heat stabilization inactivates proteolytic enzymes, kinases and phosphatases before samples are homogenized in a buffer designed to ensure optimal extraction.

## Stabilizor™ Peptide Extraction Kit

Maximize recovery of water-soluble, endogenus peptides. Heat stabilization inactivates proteolytic enzymes before samples are homogenized and peptides filtered (10kDa).

Denator AB is a privately held, biotech company based in Gothenburg, Sweden.
The company develops and markets products based on the company's proprietary heat stabilization technology. By rapid heat treatment the components of tissues and fluids are preserved, enabling researchers to reveal new, biologically-relevant information. Heat stabilization stops the biological changes that begin from the moment of sampling and cause vital information to be lost or distorted. Denator aims to offer solutions that helps improve the quality of biological samples through better sample handling and preparation, enabling optimal analytical results.

Visit www.denator.com for ordering information or to find your local Denator representative.




Denator AB
Arvid Wallgrens Backe 20
SE 413 46 Göteborg
Sweden
Phone: +46 (0)31 412 890
Fax: +46 (0)31 412 840
inform@denator.com