

Stabilizer Peptide Extraction Kit

Stabilization and extraction of endogenous peptides from tissue

- Optimize recovery of water-soluble endogenous peptides
- Produce high quality samples for consistent LC-MS analysis
- Reduce time to results
 - Follow a ready to use, validated protocol – no need for optimization
 - Use standard laboratory equipment



Introduction

The Stabilizer™ Peptide Extraction Kit, used together with the Stabilizer system, addresses the challenge of maximizing recovery of endogenous peptides from tissue samples. After rapid heat stabilization to inactivate proteolytic enzymes, samples are homogenized and molecules > 10kDa are filtered away prior to LC-MS analysis. This preparation process facilitates the differentiation and identification of endogenous peptides from other degradative peptide fragments that may originate from proteolytic cleavage in the tissue or during sample handling.

Efficient recovery of neuropeptides from heat-stabilized tissue

Endogenous peptides and their precursors are found in many tissues. Neuropeptides that are produced, or have their action, within the central nervous system (CNS) are typical endogenous

peptides, originating from neuropeptide-precursors stored in cellular vesicles. These precursors are cleaved or modified by enzymes before being released outside the cell as signals.

Results showing an increased recovery of neuropeptides from tissue samples when utilizing heat stabilization followed by an optimal extraction protocol are presented overleaf. Denator would like to thank Dr.Colgrave and her co-authors for permission to show this data that originates from their publication: *Neuropeptide profiling of the bovine hypothalamus: Thermal stabilization is an effective tool in inhibiting post-mortem degradation.* Michelle L. Colgrave, Li Xi, Sigrid A. Lehnert, Traute Flatscher-Bader, Henrik Wadensten, Anna Nilsson, Per E. Andren and Gene Wjiffels; *Proteomics* 2011, 11, 1-13.

Increased recovery of neuropeptides from bovine brain tissue

Data in Table 1 demonstrates increased recovery of endogenous neuropeptides extracted from bovine brain tissue samples after heat-stabilization.

	Heat-stabilized tissue		Frozen tissue	
	Sample 1	Sample 2	Sample 1	Sample 2
Neuropeptide-encoding precursors (99%)	14	14	8	7

Table 1: Conventional protein identification criteria were applied when identifying neuropeptide precursors i.e. a minimum of two peptides derived from the same 'protein' identified with 95% confidence, hence any precursors containing only one peptide may be missed.

Elimination of interference from degradation fragments during LC-MS analysis

Results from the MS-MS identification of all peptides (Table 2) show that heat-stabilized tissue samples contained significantly fewer peptide fragments than frozen tissue. The majority of peptides in the frozen tissue were fragments of abundant structural proteins, catalytic enzymes, blood-related proteins,

transmembrane proteins and heat-shock proteins, confirming that heat-stabilization had prevented sample degradation. By eliminating interference from protein degradation fragments, a significantly greater number of candidate neuropeptides were identified.

	Heat-stabilized tissue		Frozen tissue	
	Sample 1	Sample 2	Sample 1	Sample 2
All peptides (95%)	221	230	344	550
Candidate neuropeptides (95%)	75	103	51	33

Table 2. Identification of all peptides with 95% confidence

Product specifications

The Stabilizer Peptide Extraction Kit includes Maintainor™ Tissue cards for tissue samples, solutions, accessories and a detailed protocol optimized for sample preparation prior

to LC-MS. The protocol has been validated for LC-MS, although final samples may be in a condition suitable for other analytical techniques.

Stabilizer Peptide Extraction Kit for processing 12 tissue samples:		Additional equipment required
12 x Maintainor Tissue cards		Stabilizer system
12 x Spin filters, 10kDa cut-off		Adjustable pipette (1000µl)
Stabilization and extraction protocol		Analytical balance
3 x 45 ml Extraction fluid		Homogenization device
12 x tubes		Microcentrifuge with 1.5 ml microcentrifuge tubes
Shelf life: 12 months from production date	Storage: room temperature	

Ordering information

Stabilizer Peptide Extraction Kit

Order code: DKT 0001

Stabilizer system

Order code: DST 0001

Please contact your local Denator representative for further information.