Microwave Enhanced Enzymatic Digestion

Jonathan Collins, CEM Corporation, 3100 Smith Farm Road, Matthews, NC, www.cem.com

Microwave energy has been shown a powerful tool of enhancing enzymatic digestion of proteins. Higher efficiency digestion is obtained in 15-minutes compared to conventional digestion at 37°C for 8-hours to overnight. The method has been applied successfully with solution and in-gel samples and is compatible with a range of methods including trypsin, Lys-C, and formic acid.

A microwave system is available for processing up to 14-samples in parallel using eppendorf tubes. The vessel holder is specially designed with a microwave bath solution that ensures homogenous microwave power and even temperature control for multiple small volume samples. This allows for fast and highly efficient protein digestion that can accommodate both low and high-throughput needs.

Standard and MAPED in-gel preparation, digestion, and peptide extraction protocols[1]

Protocol	Solution	Standard, sample 6 **Hours**	MAPED, sample 7 **min**
Reduce	10 mM DTT	1	0
Alkylate	55 mM iodoacetamide	0.5	0
Dehydrate	100% ACN	0.5	5
Rehydrate	0.5 µM trypsin, 25 mM ammonium bicarbonate	0.5	5
Digest	0.5 µM trypsin, 25 mM ammonium bicarbonate	16	5
Extract	5% formic acid, 50% ACN	0.5 x 2	5 x 2
Total time		**19.5**	**25**

The identified proteins from five yeast lysate bands by 1D LC-ESI-MS with standard and MAPED in-gel preparation, digestion, and peptide extraction protocols[1]

Protein name	Molecular mass kDa	pI	Number of S/MS spectra Standard	MAPED
Pyruvate kinase	54.9	7.57	22	46
Pyruvate decarboxylase	61.7	5.76	4	4
Glyceraldehyde-3-phosphate dehydrogenase 3	35.9	6.51	2	5
Enolase I	46.9	6.16	3	8
Glyceraldehyde-3-phosphate dehydrogenase 3	35.9	6.51	1	4
Enolase I	46.9	6.16	17	24
Enolase	47.0	5.61	9	12
O-Acetylhomoserine-O-acetylserine sulfhydrylase	48.7	5.97	9	9
Alcohol dehydrogenase	37.3	6.22	3	4
Enolase I	46.9	6.16	2	4
Pyruvate decarboxylase	61.7	5.76	2	3
Glyceraldehyde-3-phosphate dehydrogenase 3	35.9	6.51	1	4
Pyruvate kinase	54.9	7.57	0	5
Heat shock protein of HSP70 family, cytoplasmic	69.9	4.84	0	3
Alcohol dehydrogenase	37.3	6.22	12	16
Alcohol dehydrogenase II	37.2	6.28	6	6
Enolase I	46.9	6.16	3	3
Glyceraldehyde-3-phosphate dehydrogenase 3	35.9	6.51	3	2
Enolase	47.0	5.61	0	4

Improved Sequence Coverage (%) in various solvent systems with Microwave Irradiation[2]

Proteins	H_2O		50% CH_3OH		30% CH_3CN	
	w/ mw	w/o mw	w/ mw	w/o mw	w/ mw	w/o mw
Myoglobin	73	76	100	76	700	66
Cytochrome c	89	73	79	79	89	75
Lysozyme	53	33	55	51	71	30
Ubiquitin	100	62	100	100	86	64

Conventional: 6-hours at 37°
Microwave: 10-minutes at 60°

10µm protein concentrations
1:25 - protease:enzyme
pH = 8 (adjusted with acetic acid or ammonium hydroxide)

References

1 Sun et al. Mol Cell. Proteomics 5, 769-776 2006.

2 Hua et al. Proteomics 6, 586-591 2006.

Conclusions

Microwave Energy represents a valuable new tool for enhancing efficiency of enzymatic digestion reactions. The new capability to digest up to 14 different samples in parallel should offer benefit to many researchers..