

## PTM-works – PTM Profiling plus

MS Bioworks PTM Profiling plus service (MSB-10) employs three enzymes to help provide both complete sequence coverage of the target protein and multiple pieces of evidence for PTM localization allowing for greater confidence in site mapping. This example below shows phosphosite mapping of human MAPK3. Here, the protein was separated on three lanes of an SDS-PAGE gel. The three bands were excised and digested with trypsin, chymotrypsin and elastase. Each digest was analyzed by LC/MS/MS on an Orbitrap Velos Pro tandem mass spectrometer. Data were searched using Mascot and search results processed using Scaffold and Scaffold PTM.

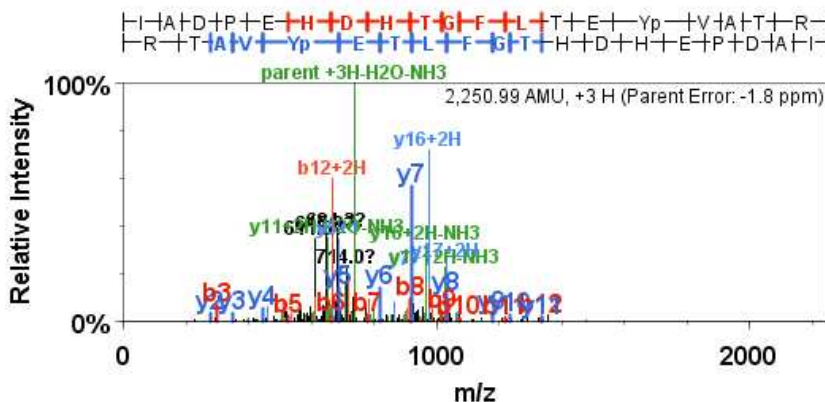
The target protein was observed with 100% sequence coverage when the data from all three enzymes was combined:

GST-MAPK3 (100%), 69,796.2 Da  
GST-MAPK3  
723 unique peptides, 1247 unique spectra, 2211 total spectra, 609/609 amino acids (100% coverage)

MSPILGYWKI	KGLVQPTRL	LEYLEEKYEE	HLYERDEGDK	WRNKKFELGL	EFPNLPYYID
GDVKLTQSM	IIRYIADKHN	MLGGCPKERA	EISMLEGAVL	DIRYGVSRIA	YSKDFETLKV
DFLSKLPEML	KMFEDRLCHK	TYLNGDHVTH	PDFMLYDALD	VVLYMDPMCL	DAFPKLVCFK
KRIEAIPIQID	KYLKSSKYIA	WPLQGWQATF	GGGDHPPKSD	LVPRGSPGIP	MAAAAAQGGG
GGEPRRTTEGV	GPGVPGVEVM	VKGQPFQVGP	RYTQLQYIGE	GAYGMVSSAY	DHVRKTRVAI
KKISPFHQIT	YCRRTLREIQ	ILLRFRHENV	IGIRDILRAS	TLEAMRDVYI	VQDLMETDLY
KLLKSQQLSN	DHICYFLYQI	LRGLKYIHSA	NVLHRDLKPS	NLLINTTCDL	KICDFGLARI
ADPEHDHTGTF	LTEYVATRWY	RAPEIMLNSK	GYTKSIDIWS	VGCILAEMLS	NRPIFPKHY
LDQLNHILGI	LGSPSQEDLN	CINMKARNY	LQSLPSKTKV	AWAKLFPKSD	SKALDLLDRM
LTFNPNKRIT	VEEALAHPYL	EQYYDPTDEP	VAEEPFTFAM	ELDDLPKERL	KELIFQETAR
FQPGVLEAP					

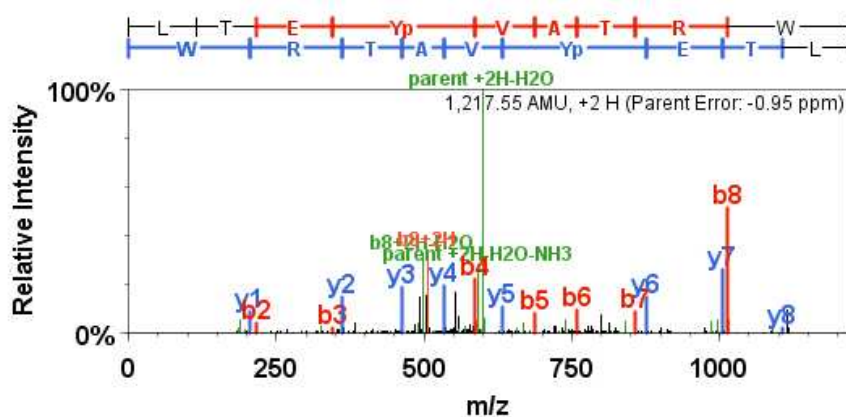
Five sites of phosphorylation were detected. The known Tyr187 site was validated following observation with all three enzymes:

**Trypsin – IADPEHDHTGFLTE(pY)VATR**



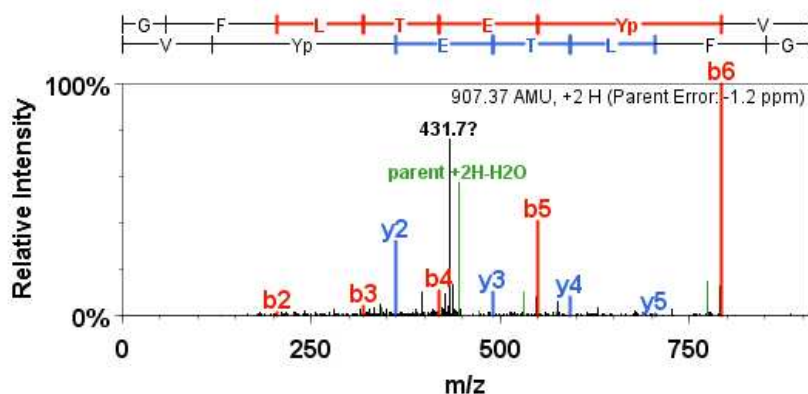
Note poor fragmentation around pTyr. The A-score (from Scaffold PTM) is 21.77 and although site localization probability is 100% this is not the highest quality spectrum.

**Chymotrypsin – LTE(pY)VATRW**



Note complimentary y- and b-ion pairs around pTyr. The A-score (from Scaffold PTM) is 52.75 and site localization probability 100%.

**Elastase – GFLTE(pY)V**



Note the abundant b5 and b6 ions that span pTyr. The A-score (from Scaffold PTM) is 45.27 and site localization probability 100%.

This powerful approach to PTM localization is best suited to targeting individual proteins, our methods are compatible with most buffers and we recommend submitting at least 1µg of material, in gel or solution. MS Bioworks PTM Profiling plus service can be ordered directly from our website at [www.msbioworks.com](http://www.msbioworks.com).

Service ID	Cost (\$)
MSB-10	900