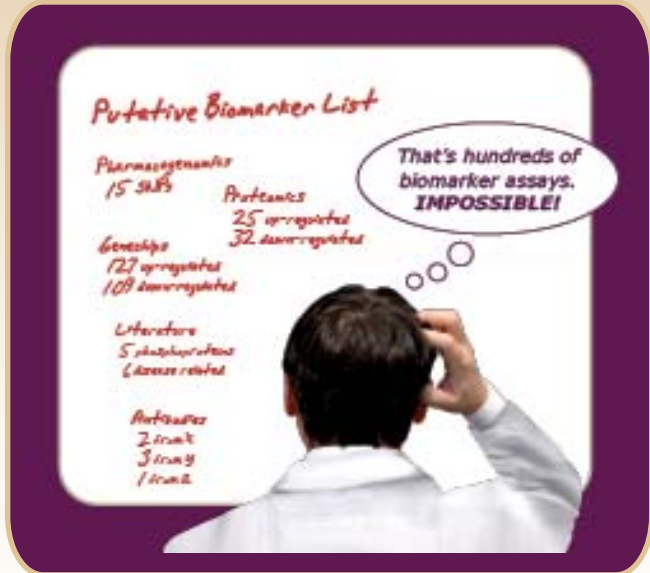


assayexpress™ ◇ Biomarker Assay Development Service

Biomarker assay development has been a bottleneck for many years. The time to produce and test antibodies is long and resource intensive with a low success rate. NextGen Sciences develops mass spectrometry-based protein assays employing multiple reaction monitoring (MRM).

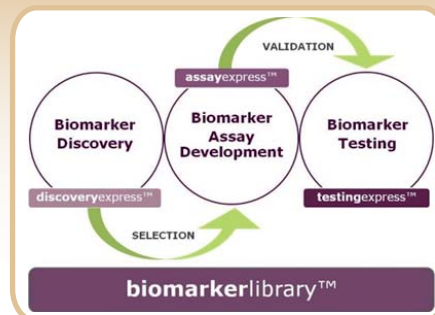


assayexpress™ Features:

- Mass spectrometry based assay platform
- Assay development time is weeks to months
- Optimized for multiplexing, short run times and lowest sample requirement
- Quantification is not dependent on affinity reagents
- Absolute and relative quantification
- Easily tailored across species
- Empirical selection of peptides for MRM assay
- High specificity for targeted protein(s) including post-translational modifications and isoforms

biomarkerexpress™ services

NextGen Sciences, Inc has developed a suite of biomarker services to significantly decrease timelines and increase the success rates traditionally associated with the various stages of biomarker development.



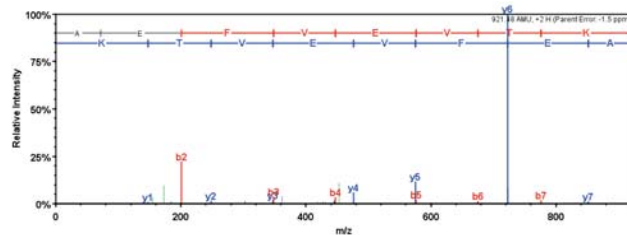
Technology Overview of Biomarker Assay Development using MRM

Multiple Reaction Monitoring (MRM) is a mass spectrometry-based assay historically used for small molecule quantitation. Whereas an intact protein is too large for MRM, proteolytically generated peptides can easily be measured. There are three key steps in developing a protein assay using the MRM technique.

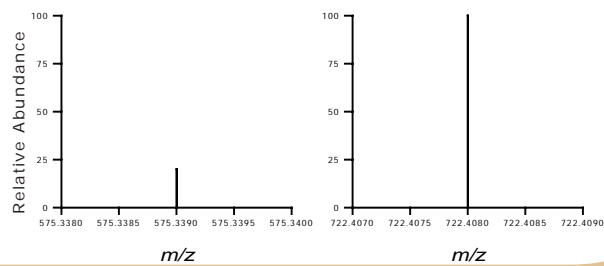
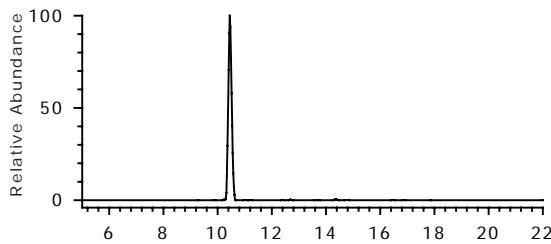
Selection of Peptides. Typically 2 to 3 peptides are selected from experimental data as surrogates for each of the targeted proteins. The selected peptides possess unique sequences and fragment ion signatures to achieve specificity.

```

MKWVTFISLL LLFSSAYSRG VFRRDTHKSE IAHRFKDLGE EHFKGLVLIA FSQYLQQCFP
DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK VASLRETYGD MADCEKQEP
ERNECFLSHK DSDPDLPLK PDPNTLCDEF KADEKKFWGK YLYEIARRHP YFYAPELLEY
ANKYNGVFQE CQQAEDKGC LLFKLETMRE KVLASSARQR LRCASIQKFG ERALKAWSVA
RLSQKFPKAE FVEVTELVTD LTKVHKECCH GDLLCADDR ADLAKYICDN QDTISSKLKE
CCDKPLLEKS HCLAEVEKDA IPENLPLTA DPAEDKDVCK NYQEAKDAPL GSFLYEYSRR
HPEYAVSVLL RLAKYEATL EECCKDDPH ACYSTVDFKL KHLVDEPNL IKQNCDFEK
LGEYGFONAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC CTKPESERMP CTEDYLSLIL
NRLCVLHEKT PVSEKVKCC TESLVNRRPC FSALTPDETY VPKAFDEKLF TFHADICTLP
DTEKQIKKOT ALVELLKHKP KATEEQKTV MENFVAFVK CCAADDEKAC FAVEGPKLVV
STQTALA
    
```



MRM Assay Optimization. Candidate peptides are tested in the relevant sample. Chromatographic and mass spectrometer conditions are optimized so that the targeted proteins can be monitored with the least amount of sample and the shortest assay run time. Sample preparation conditions are optimized as necessary to observe lower abundance proteins.



Protein Quantification. The MRM approach can be used to yield absolute or relative protein biomarker quantification data. Heavy isotope labeled peptide standards are used for absolute quantification. Calibration curves are constructed based on the ratio of the labeled and unlabeled peptides in a complex matrix representative of the samples. Intra-assay reproducibility is also established.

