

Assay Development Working Group - Experiments for Assay Characterization

Experiment 1: *Response Curve*

- Development of multipoint response curve (1 blank and a minimum of 6 concentration points).
- Samples prepared in digested matrix background (i.e. plasma, tissue, cells, etc).
- Used for the determination of LOD, LLOQ and linearity.
- Multiple replicates analyzed.



Experiment 2: *Mini-Validation of Repeatability*

- Examines intra- and inter-assay variability.
- Uses the LLOQ from Experiment 1 from which 3 concentrations (Low, Medium and High) are used to assess repeatability.
- 3 replicates processed and measured on 5 different days.



Experiment 3: *Selectivity*

- Examines the response of a peptide in six different biological replicates of the matrix.
- Replicates analyzed with no spike and 1/3 the Medium and Medium concentrations defined in Experiment 2.



Experiment 4: *Stability*

- Examines the stability of a peptide spiked into a background matrix
- Stability assessed based on peak area variability following:
 - different storage conditions (4C and -70C) over time.
 - freeze-thaw cycles
- Variability compared to data collected from Experiment 2.



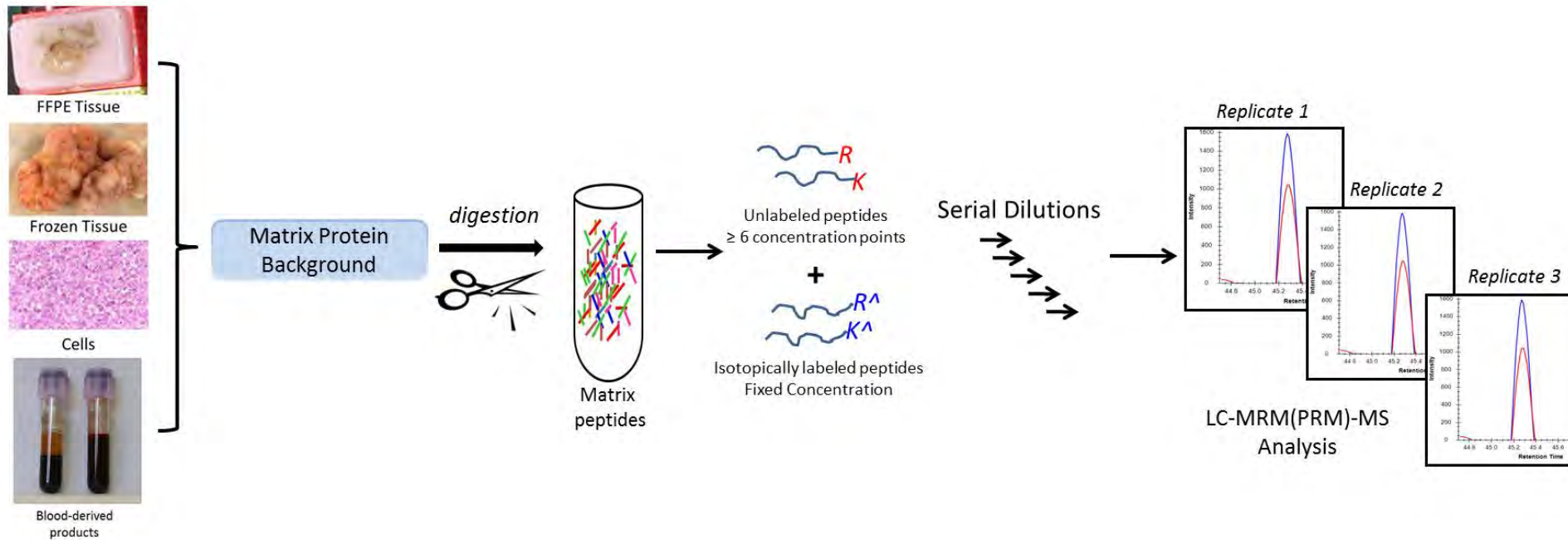
Experiment 5: *Reproducible Detection of Endogenous Analyte*

- Representative sample containing endogenous analyte is digested 5 times on each of 5 days.
- Examines intra- and inter-assay variability of the entire assay workflow, including digestion.

Experiment 1: *Response Curve*

KEY POINTS

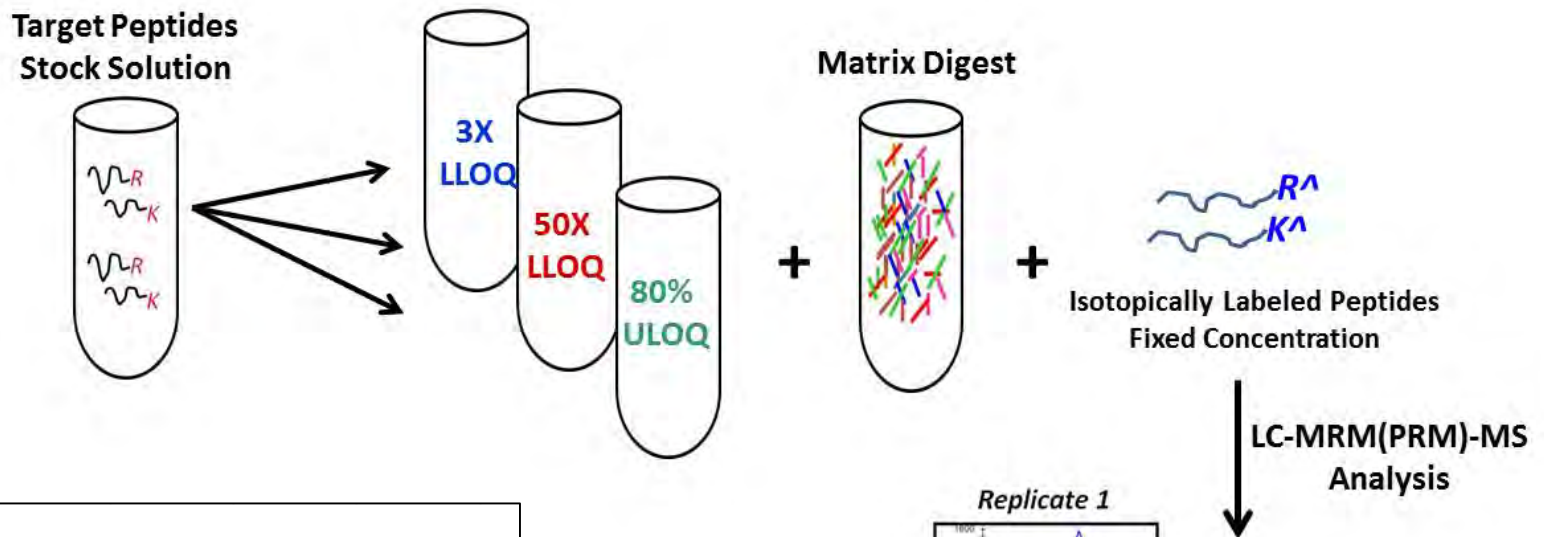
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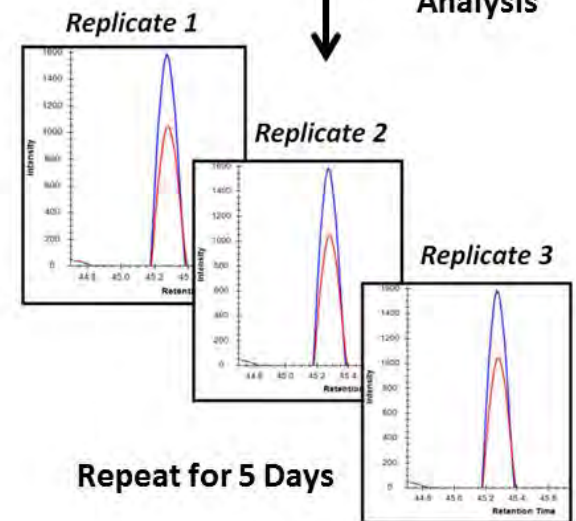
Experiment 2: *Mini-Validation of Repeatability*

KEY POINTS

- Examines intra- and inter-assay variability.
- Uses the LLOQ from Experiment 1 from which 3 concentrations (Low, Medium and High) are used to assess repeatability.
- 3 replicates processed and measured on 5 different days.



Day	Replicate			
	1	2	3	
1	2156	1989	2095	4.1%
2	2066	1653	2103	12.9%
3	1509	1755	1536	8.4%
4	1862	2132	2454	13.8%
5	1617	1515	1724	6.5%
			Ave	9.1%
	15.1%	13.8%	18.1%	15.7%
			Total error	18.1%

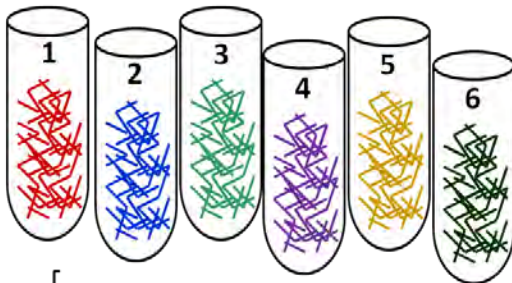


Experiment 3: *Selectivity*

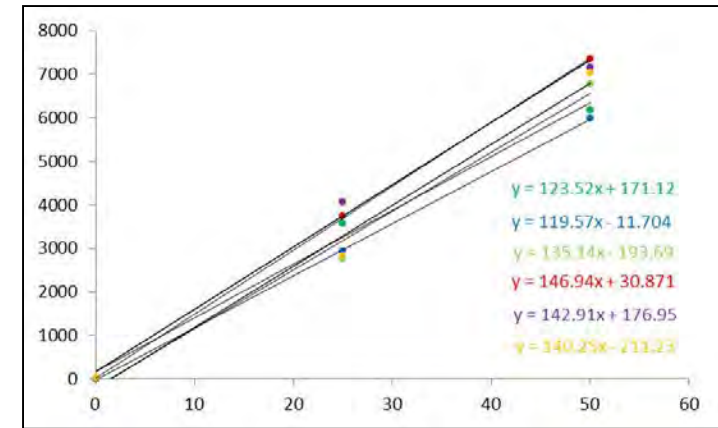
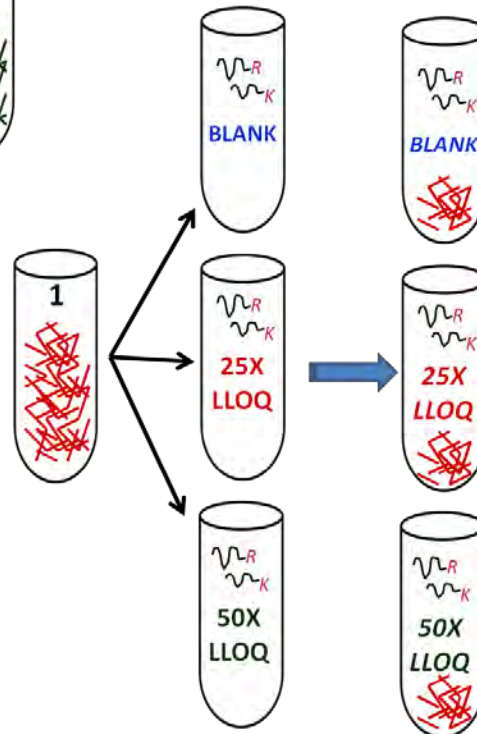
KEY POINTS

- Examines the response of a peptide in six different biological replicates of the matrix.
- Replicates analyzed with no spike and ½ the Medium and Medium concentrations defined in Experiment 2.

Six Biological Replicates of Matrix



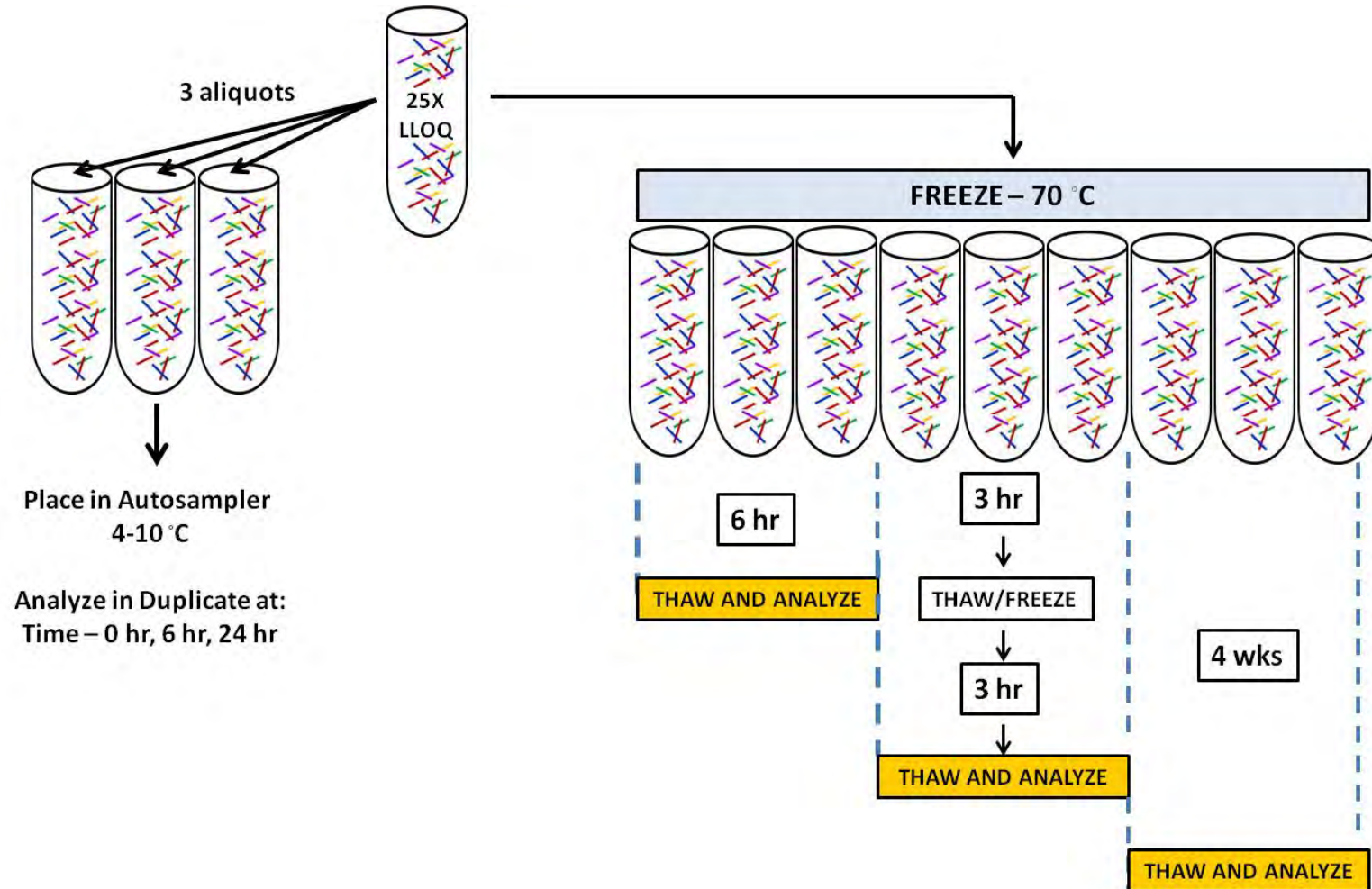
Target Peptides Stock Solutions



Experiment 4: *Stability*

KEY POINTS

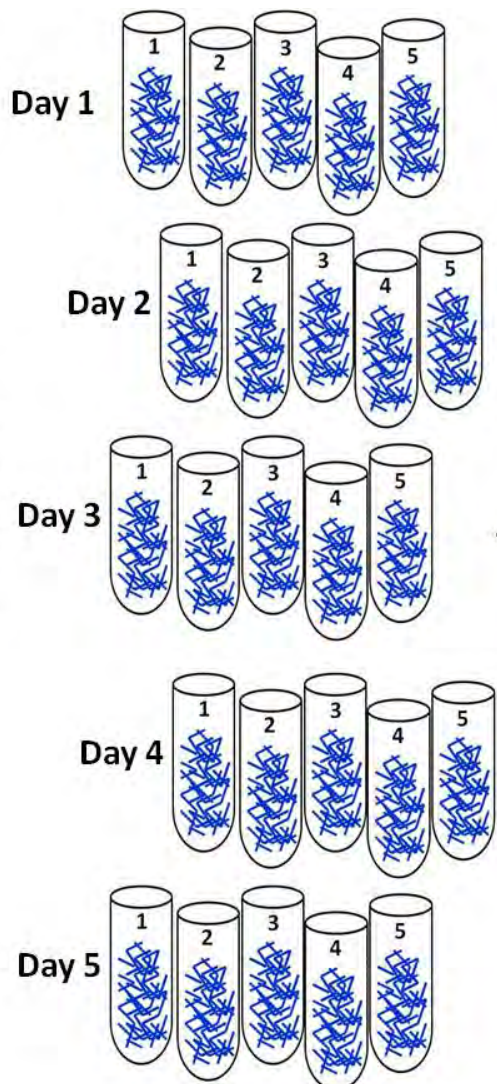
- Examines the stability of a peptide spiked into a background matrix
- Stability assessed based on peak area variability following:
 - different storage conditions (4C and -70C) over time.
 - freeze-thaw cycles
- Variability compared to data collected from Experiment 2.



Experiment 5: *Reproducible Detection of Endogenous Analyte*

KEY POINTS

- Representative sample containing endogenous analyte is digested 5 times on each of 5 days.
- Examines intra- and inter-assay variability of the entire assay workflow, including digestion.



LC-MRM(PRM)-MS
Analysis

Day	Replicate					
	1	2	3	4	5	
1	19396	17334	17325	15488	16573	8.3%
2	24686	21386	21000	23260	22226	6.6%
3	22121	26532	22050	20064	26730	12.7%
4	17280	18572	20295	14404	21384	14.8%
5	21881	19859	21450	16840	22113	10.7%
	13.4%	17.2%	9.0%	20.1%	16.5%	
						Ave 10.6%
						13.2%
						Total error 17.0%