

# 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.



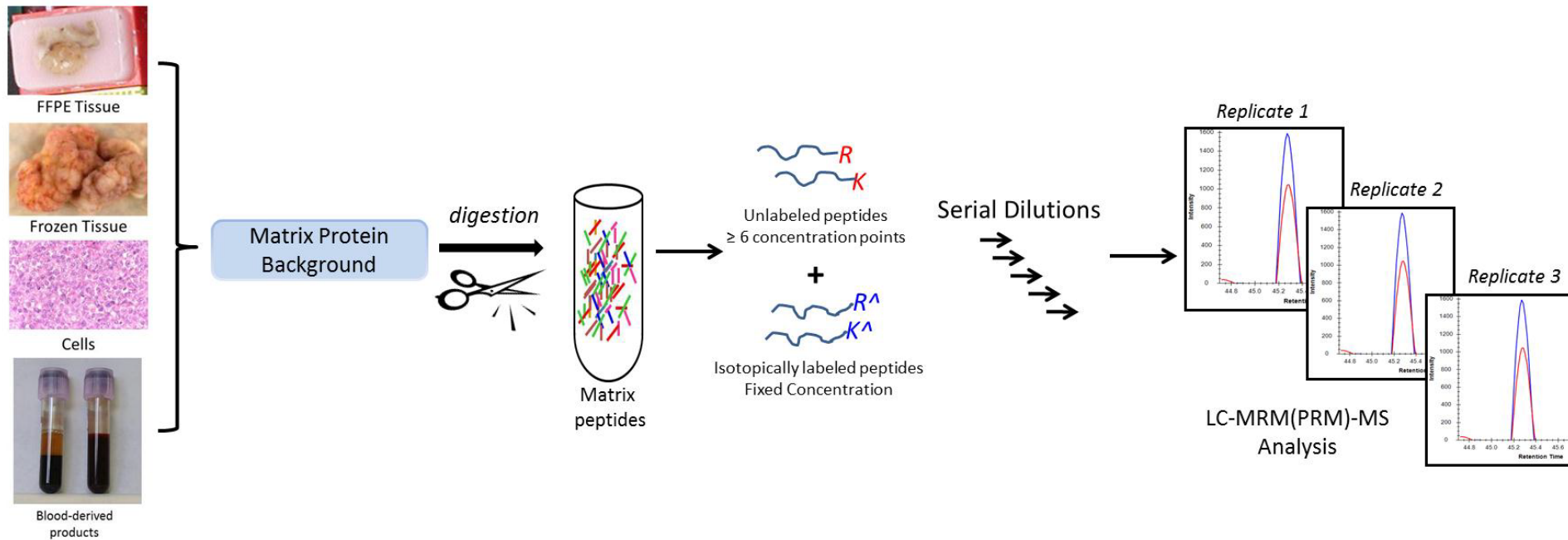
## Experiment 5: *Reproducible Detection of Endogenous Analyte*

- 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

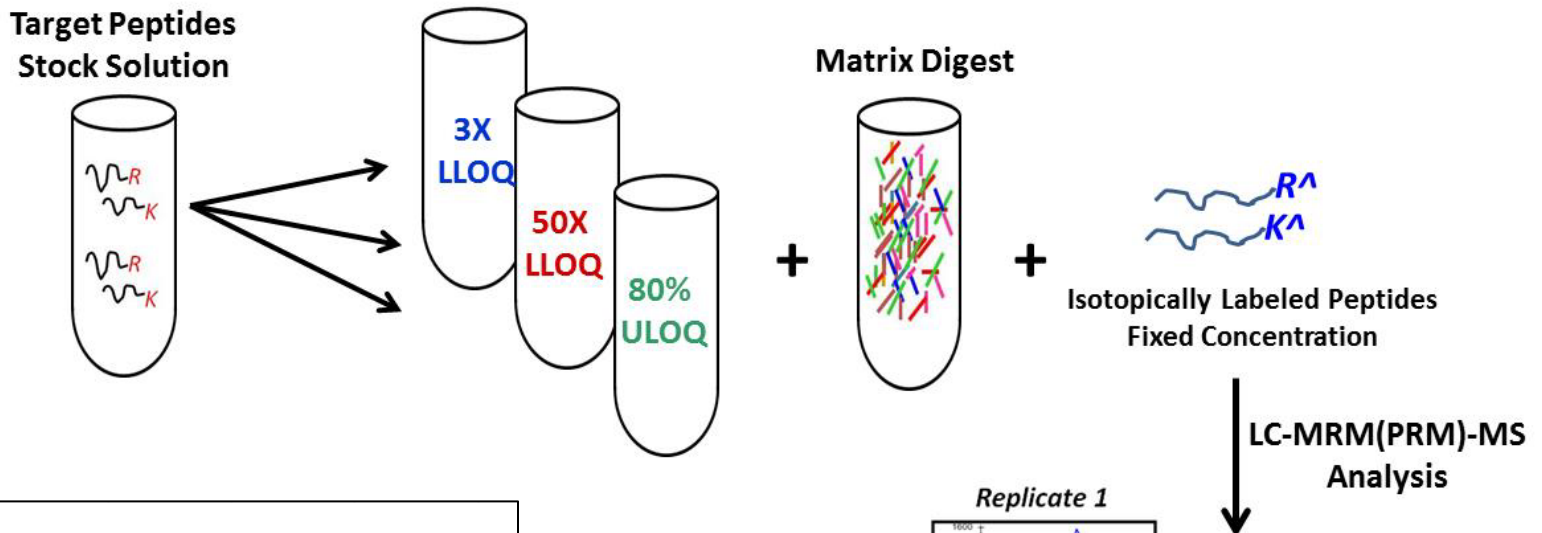
- Development of multipoint response curve (1 blank and a minimum of 6 concentration points).
- Samples prepared in digested matrix background (i.e. plasma, tissue, cells, etc).
- Used for the determination of LOD, LLOQ and linearity.
- Multiple replicates analyzed.



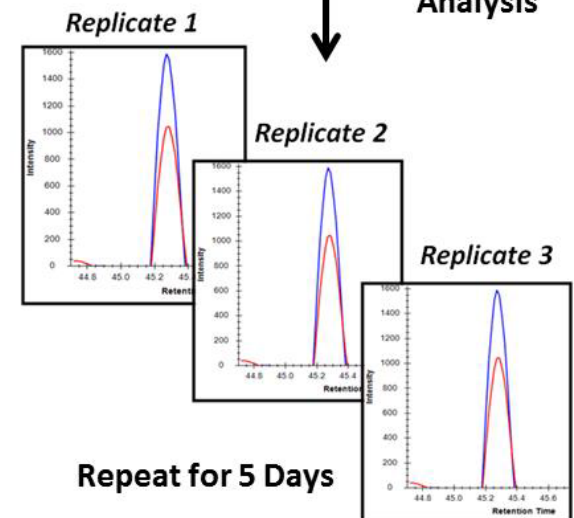
## Experiment 2: *Mini-Validation of Repeatability*

## 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.



	Replicate					
Day	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%		
				<table><tr><td>Ave</td><td>9.1%</td></tr></table>	Ave	9.1%
Ave	9.1%					
	15.1%	13.8%	18.1%	<table><tr><td>15.7%</td><td></td></tr></table>	15.7%	
15.7%						
				<table><tr><td>Total error</td><td>18.1%</td></tr></table>	Total error	18.1%
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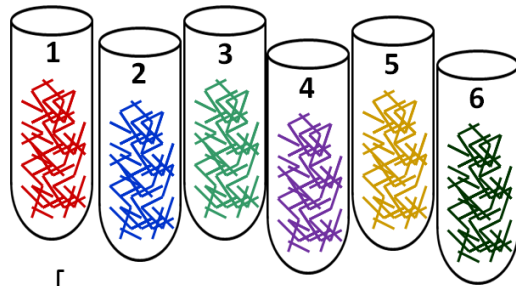


## Experiment 3: *Selectivity*

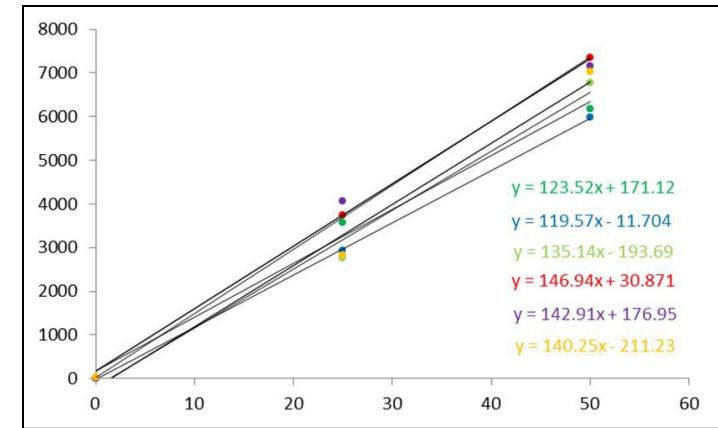
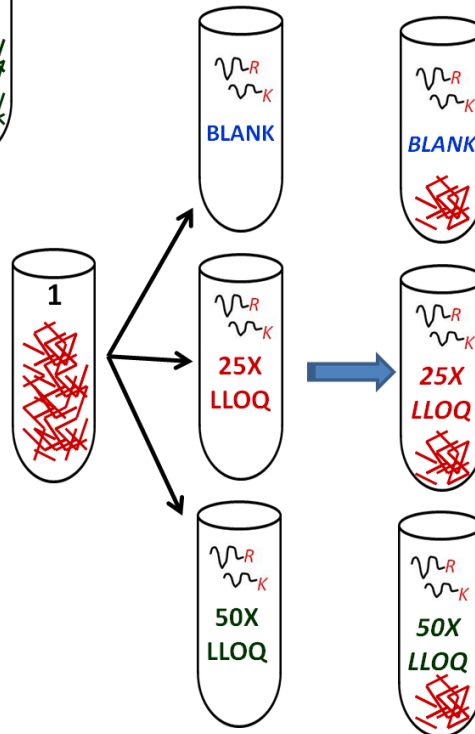
### KEY POINTS

- 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



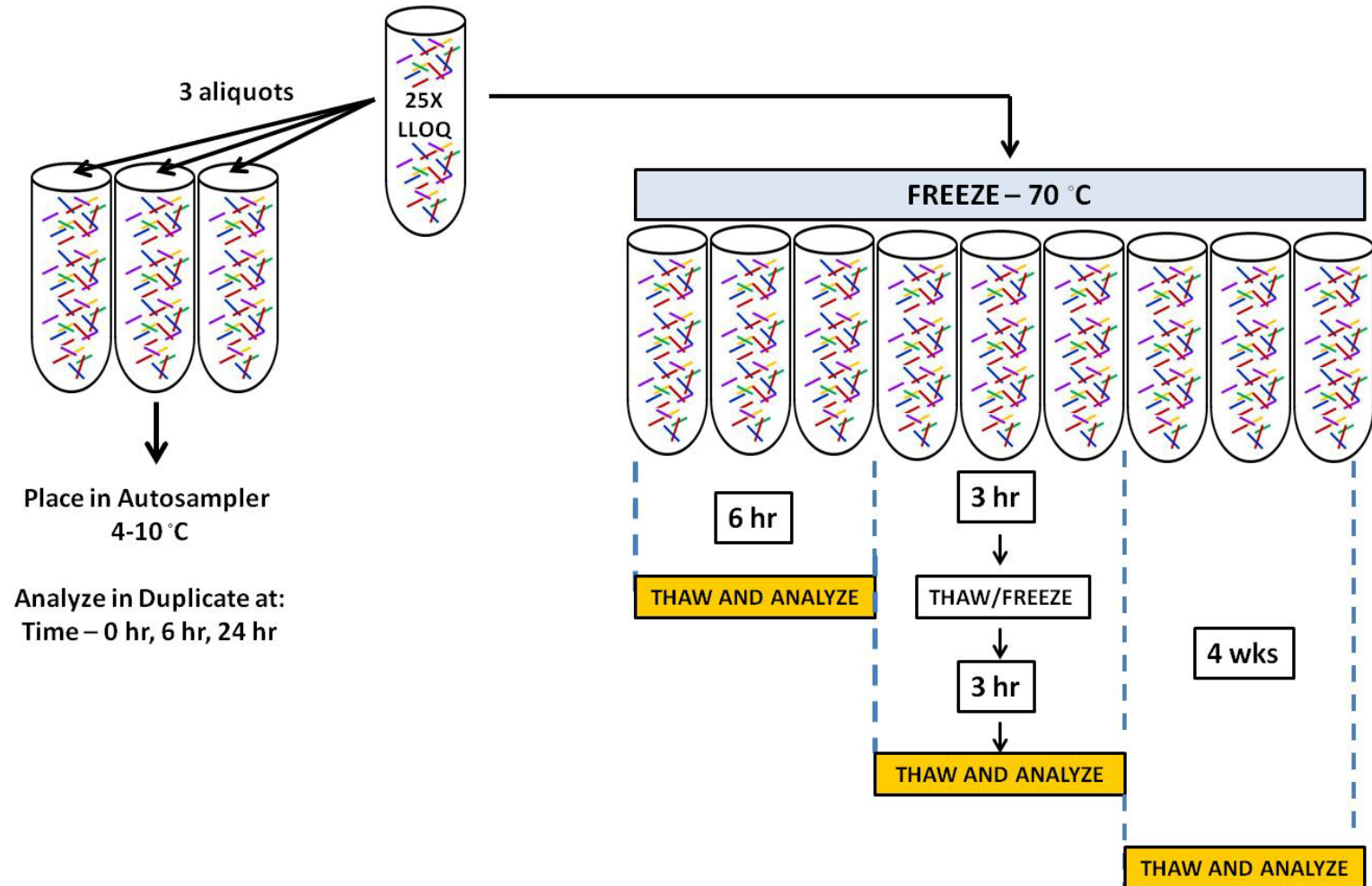
### Target Peptides Stock Solutions



## 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.

