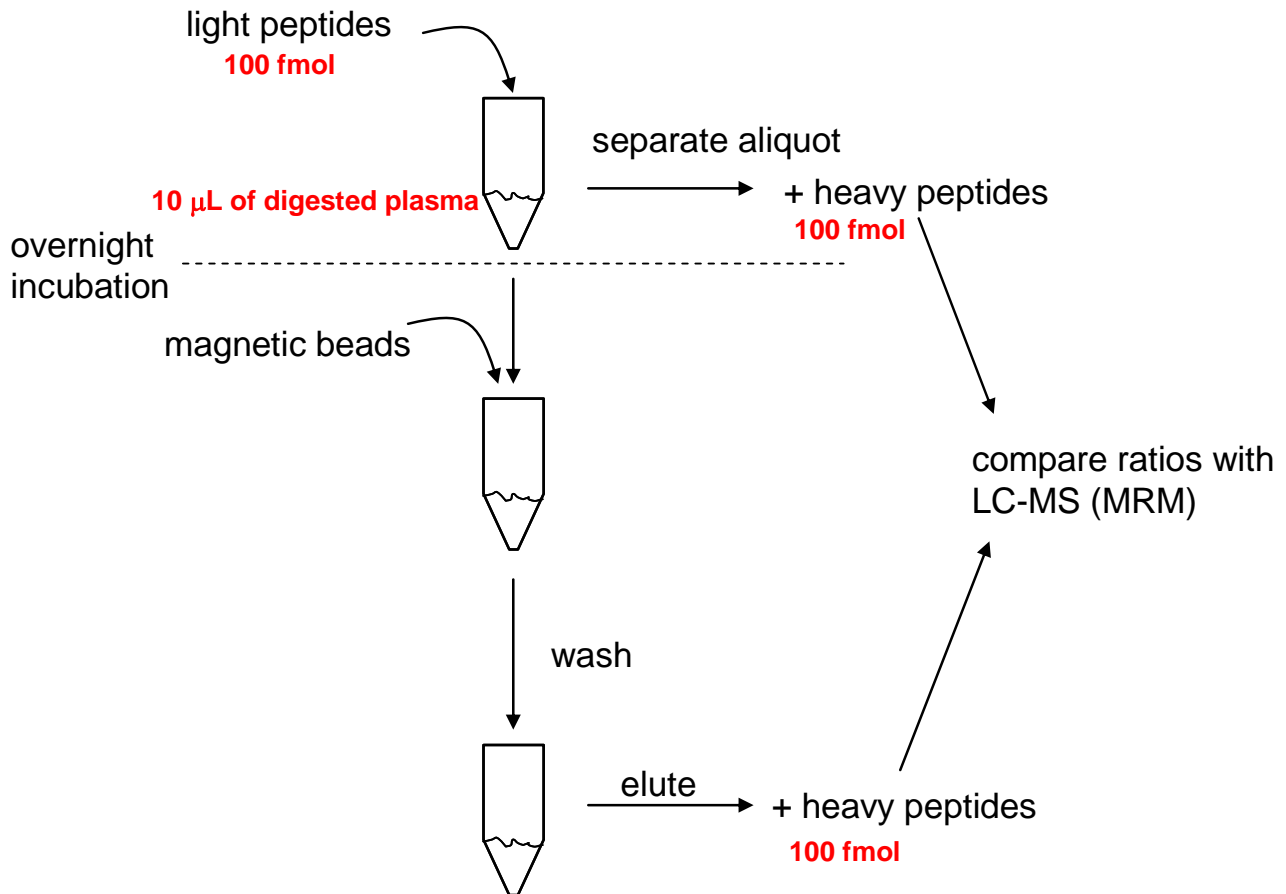


Supplemental Table 1. Peptide transitions and optimum parameters determined on a 4000 QTRAP mass spectrometer.

Description	Target peptide sequence	MRM Transitions (light)		MRM Transitions (heavy)		DP	CE	Fragment Ion Type	Rank
		Q1	Q3	Q1	Q3				
L-plastin	YTLNILEDIGGGQK	760.9	559.3	764.9	567.3	100	48	y6	1
		760.9	916.5	764.9	924.5	100	37	y9	2
		760.9	803.4	764.9	811.4	100	38	y8	3
Plectin 8	AGTLSITEFADMLSGNAGGFR	1058.0	678.3	1063.0	688.3	110	44	y7	1
		1058.0	765.4	1063.0	775.4	110	49	y8	2
		1058.0	878.5	1063.0	888.5	110	51	y9	3
Osteopontin	GDSLAYGLR	476.3	579.3	481.3	589.3	60	25	y5	1
		476.3	508.3	481.3	518.3	60	25	y4	2
		476.3	692.4	481.3	702.4	60	26	y6	3
Tumor Protein D52	LGISSLQEFK	561.3	838.4	565.3	846.4	80	28	y7	1
		561.3	751.4	565.3	759.4	80	28	y6	2
		561.3	1008.5	565.3	1016.5	80	28	y9	3
Hypoxia up-regulated	LYQPEYQEVSTEEQR	949.9	749.3	954.9	759.3	110	47	y6	1
		949.9	1494.7	954.9	1504.7	110	44	y12	2
		949.9	1150.5	954.9	1150.5	110	38	b9	3
Disulphide isomerase A4	VEGFPTIYFAPSGDK	814.4	1195.6	818.4	1203.6	100	34	y11	1
		814.4	884.4	818.4	892.4	100	39	y8	2
		814.4	997.5	818.4	1005.5	100	42	y9	3
Fibulin 2	IGPAPAFAGDTISLTITK	887.0	1434.8	891.0	1442.8	105	42	y14	1
		887.0	1048.6	891.0	1056.6	105	45	y10	2
		887.0	1119.6	891.0	1127.6	105	45	y11	3
Calumenin	SFDQLTPEESK	640.8	589.3	644.8	597.3	78	35	y5	1
		640.8	690.3	644.8	698.3	78	29	y6	2
		640.8	803.4	644.8	811.4	78	31	y7	3
Legumain	DYTGEDVTPENFLAVLR	970.0	1058.6	975.0	1068.6	105	48	y9	1
		970.0	1159.7	975.0	1169.7	105	43	y10	2
		970.0	881.4	975.0	881.4	105	37	b8	3

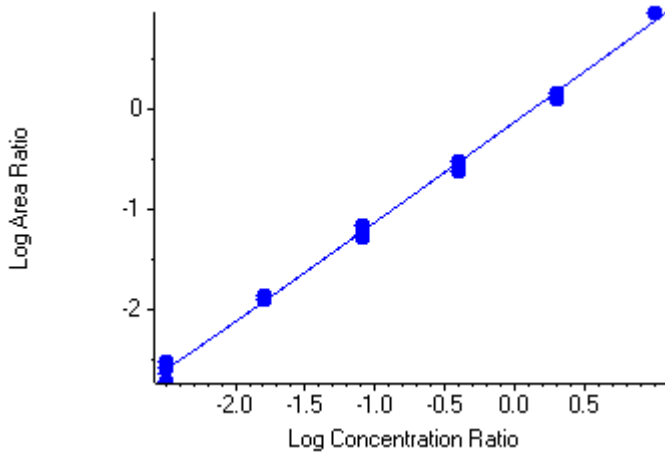
Supplemental Figure 1. Workflow for determining peptide recovery following immunoaffinity enrichment.



Supplemental Figure 2. Response curves for each analyte transition in a multiplexed assay. Heavy peptide was spiked in at varying concentrations keeping light peptide constant for use as the internal standard. The log peak area ratio is plotted versus the log peptide molar ratio. Each point is an individual measurement.

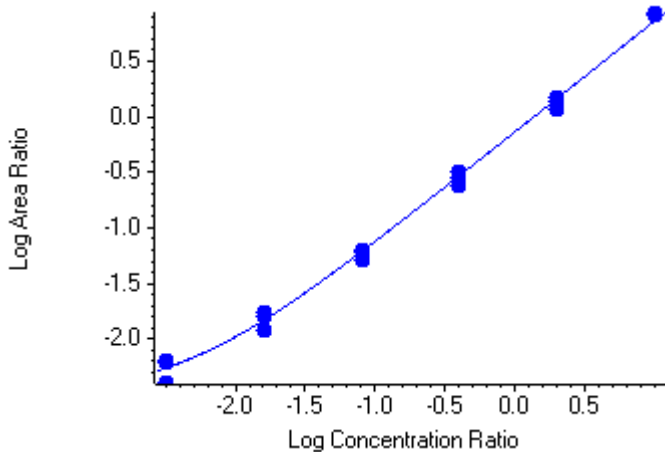
Analyte Name: Calumenin.SFDQLTPEESK.1y6.heavy

Calibration Equation: $y = 0.74340 x + 1.98673e-4$ ($r = 0.98606$) (weighting: $1 / x^2$)



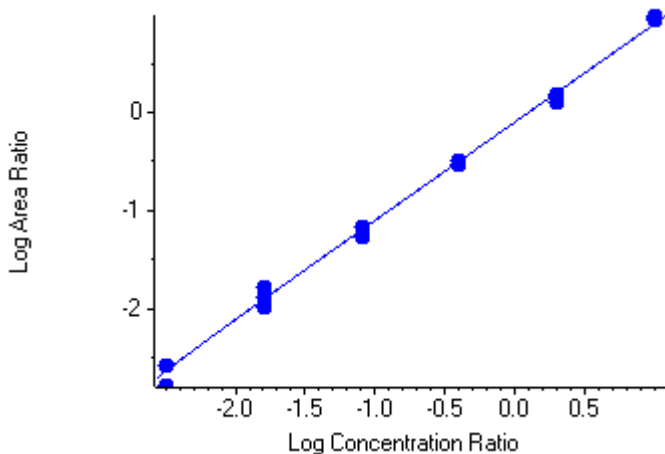
Analyte Name: Calumenin.SFDQLTPEESK.2y5.heavy

Calibration Equation: $y = 0.72787 x + 0.00320$ ($r = 0.96756$) (weighting: $1 / x^2$)



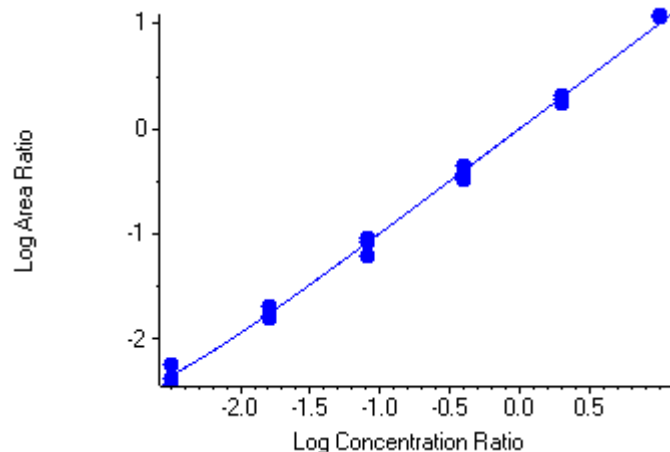
Analyte Name: Calumenin.SFDQLTPEESK.3y9.heavy

Calibration Equation: $y = 0.80295 x - 2.06589e-4$ ($r = 0.98614$) (weighting: $1 / x^2$)



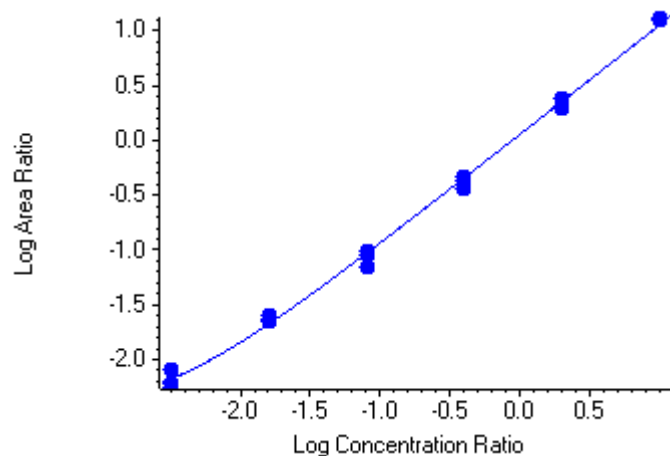
Analyte Name: DisulphideIsomeraseA4.VEGFPTIYFAPSGDK.1y11.heavy

Calibration Equation: $y = 1.01338 x + 0.00131$ ($r = 0.98268$) (weighting: $1 / x^2$)



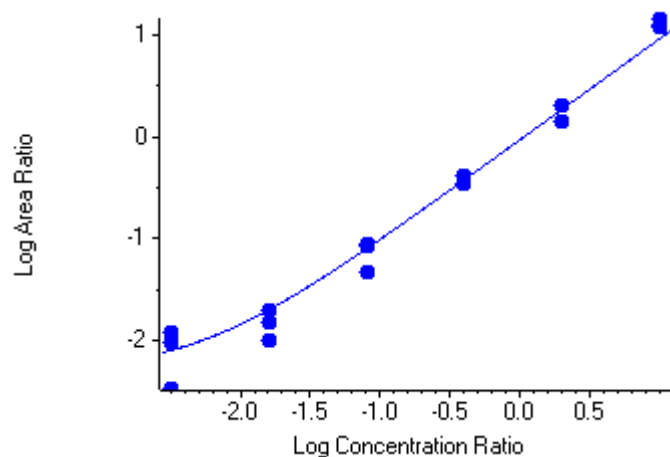
Analyte Name: DisulphideIsomeraseA4.VEGFPTIYFAPSGDK.2y8.heavy

Calibration Equation: $y = 1.13439 x + 0.00301$ ($r = 0.98041$) (weighting: $1 / x^2$)



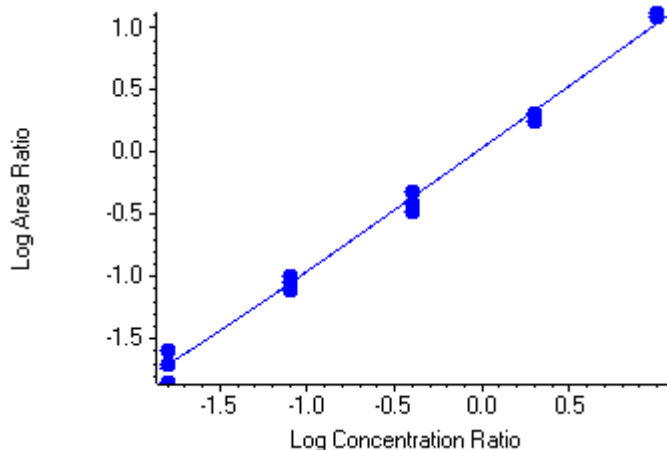
Analyte Name: DisulphideIsomeraseA4.VEGFPTIYFAPSGDK.3y13.heavy

Calibration Equation: $y = 0.93828 x + 0.00495$ ($r = 0.84204$) (weighting: $1 / x^2$)



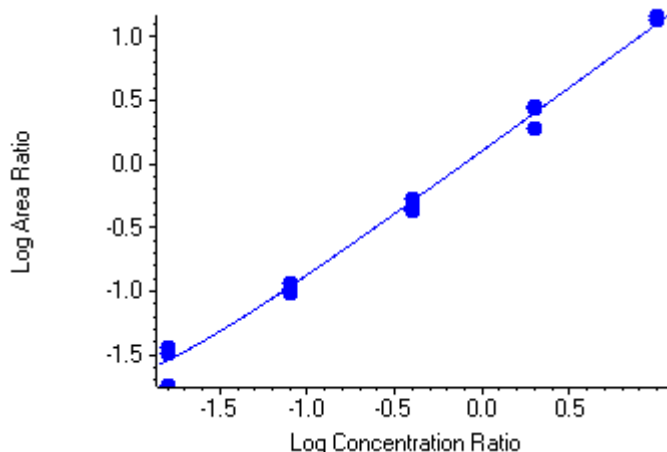
Analyte Name: Fibulin2.IGPAPAFAGDTISLTITK.1y14.heavy

Calibration Equation: $y = 1.08459 x + 0.00246$ ($r = 0.97772$) (weighting: $1 / x^2$)



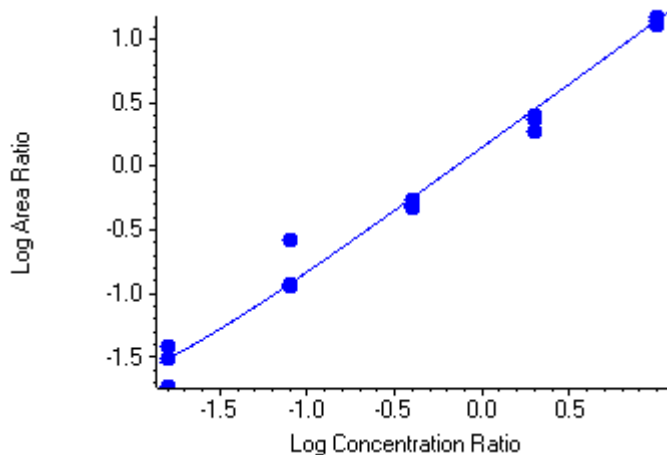
Analyte Name: Fibulin2.IGPAPAFAGDTISLTITK.2y10.heavy

Calibration Equation: $y = 1.26614 x + 0.00858$ ($r = 0.97038$) (weighting: $1 / x^2$)



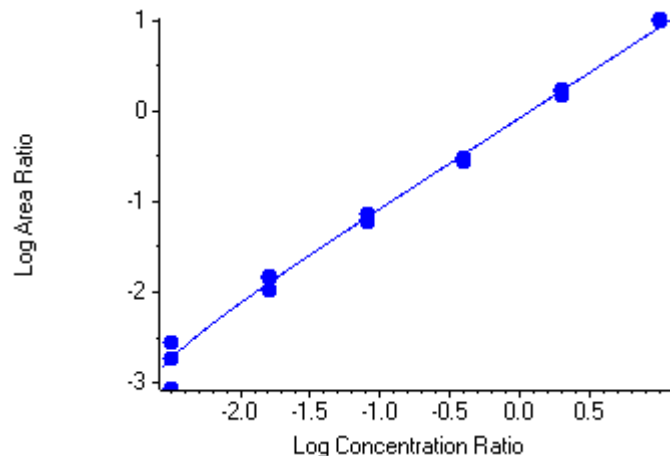
Analyte Name: Fibulin2.IGPAPAFAGDTISLTITK.3y11.heavy

Calibration Equation: $y = 1.40501 x + 0.00847$ ($r = 0.90879$) (weighting: $1 / x^2$)



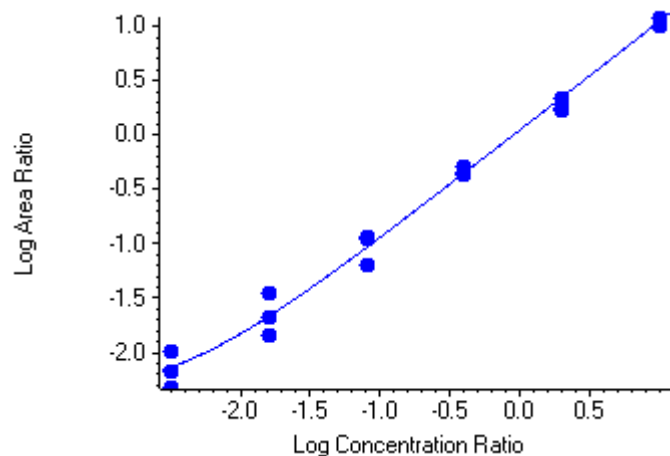
Analyte Name: HypoxiaUpregulated.LYQPEYQEVSTEEQR.1y12.heavy

Calibration Equation: $y = 0.84230 x + -8.35404e-4$ ($r = 0.97981$) (weighting: $1 / x^2$)



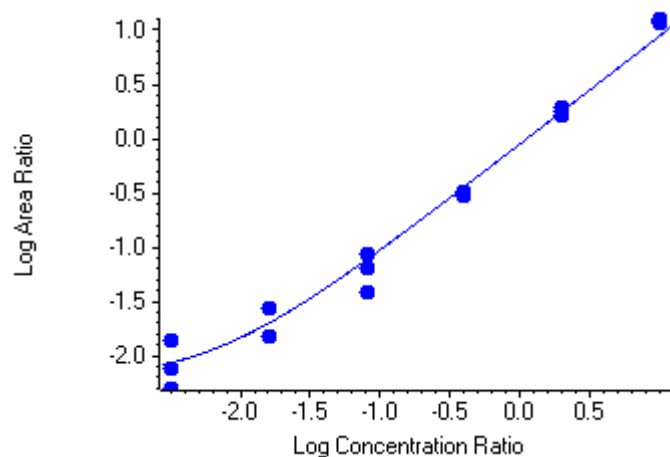
Analyte Name: HypoxiaUpregulated.LYQPEYQEVSTEEQR.2y6.heavy

Calibration Equation: $y = 1.11644 x + 0.00375$ ($r = 0.92279$) (weighting: $1 / x^2$)



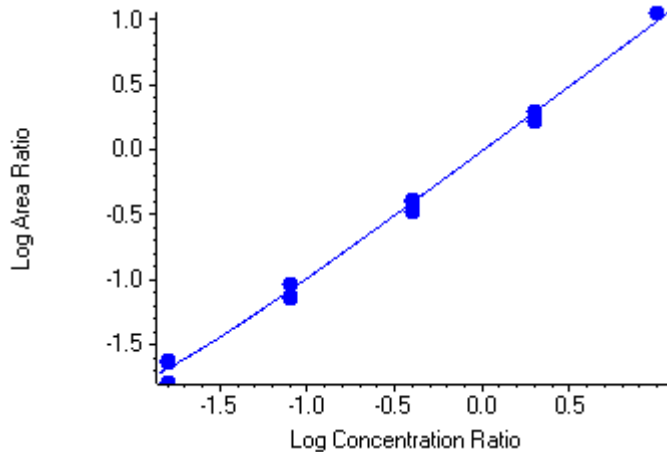
Analyte Name: HypoxiaUpregulated.LYQPEYQEVSTEEQR.3y8.heavy

Calibration Equation: $y = 0.88891 x + 0.00574$ ($r = 0.83097$) (weighting: $1 / x^2$)



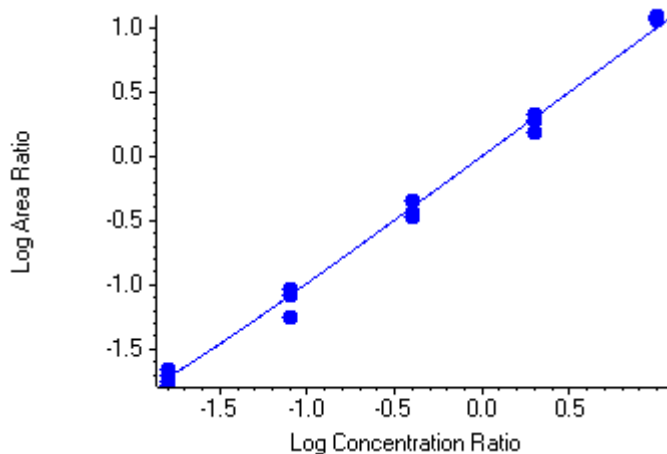
Analyte Name: Legumain.DYTGEDVTPENFLAVLR.1y9.heavy

Calibration Equation: $y = 0.97719x + 0.00521$ ($r = 0.98501$) (weighting: $1/x^2$)



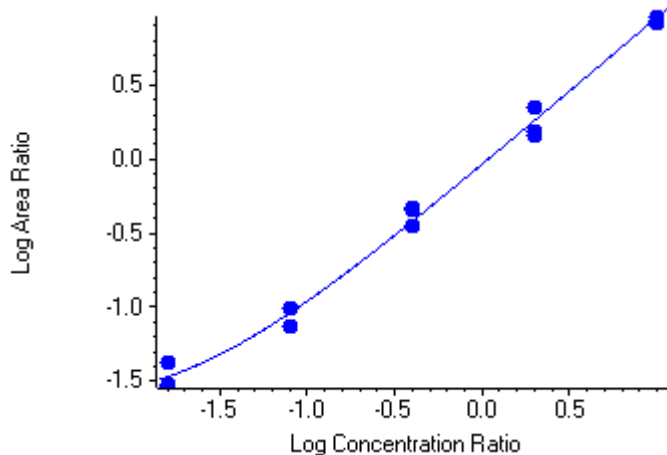
Analyte Name: Legumain.DYTGEDVTPENFLAVLR.2y10.heavy

Calibration Equation: $y = 0.99760x + 0.00306$ ($r = 0.98106$) (weighting: $1/x^2$)



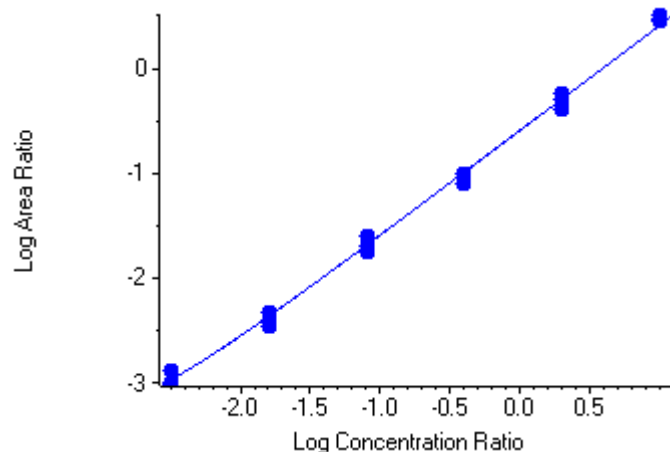
Analyte Name: Legumain.DYTGEDVTPENFLAVLR.3b8.heavy

Calibration Equation: $y = 0.90703x + 0.01938$ ($r = 0.96271$) (weighting: $1/x^2$)



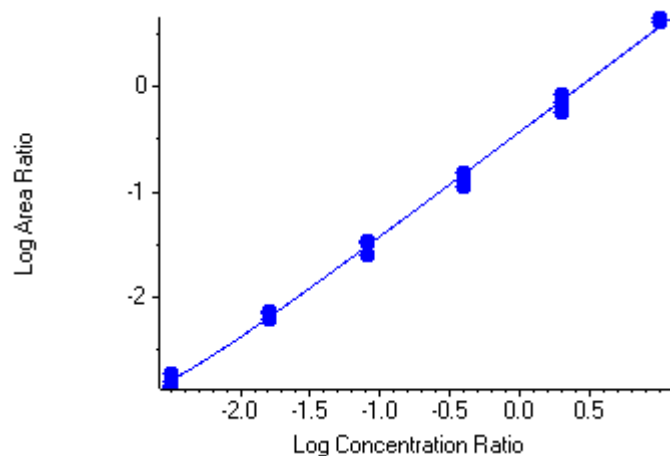
Analyte Name: Lplastin.YTLNILEDIGGGQK.1y9.heavy

Calibration Equation: $y = 0.25737 x + 2.70961e-4$ ($r = 0.98538$) (weighting: $1 / x^2$)



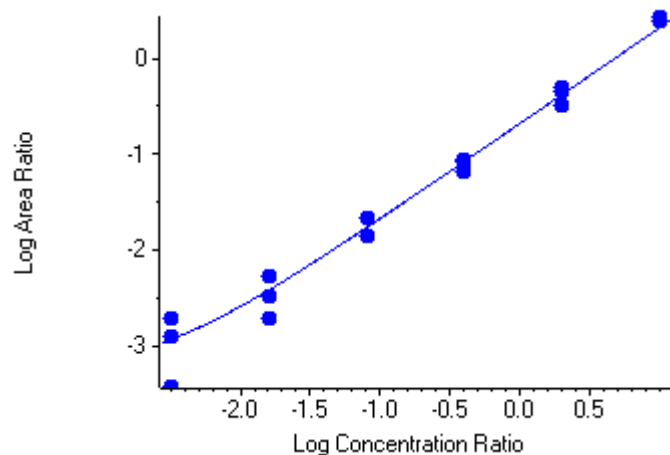
Analyte Name: Lplastin.YTLNILEDIGGGQK.2y8.heavy

Calibration Equation: $y = 0.37357 x + 4.15095e-4$ ($r = 0.98519$) (weighting: $1 / x^2$)



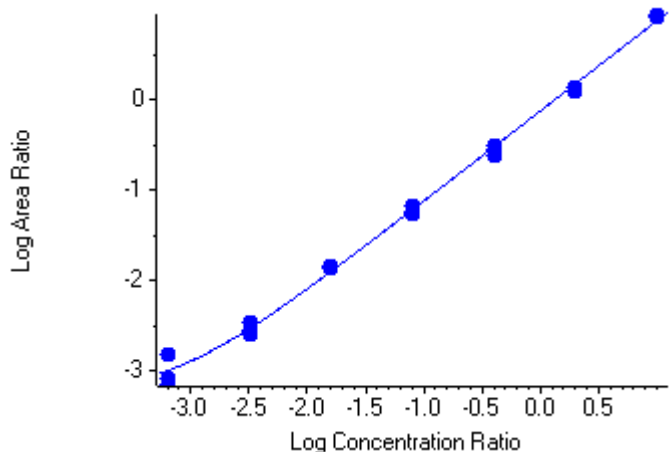
Analyte Name: Lplastin.YTLNILEDIGGGQK.3y10.heavy

Calibration Equation: $y = 0.21032 x + 5.14210e-4$ ($r = 0.88814$) (weighting: $1 / x^2$)



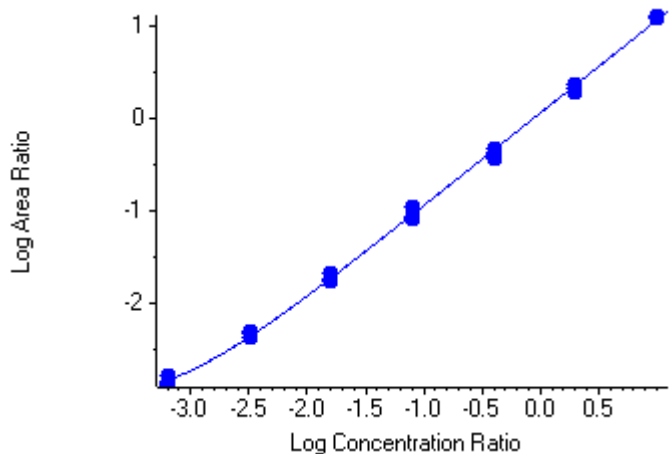
Analyte Name: Osteopontin.GDSLAYGLR.1y5.heavy

Calibration Equation: $y = 0.75796 x + 5.15684e-4$ ($r = 0.94770$) (weighting: $1 / x^2$)



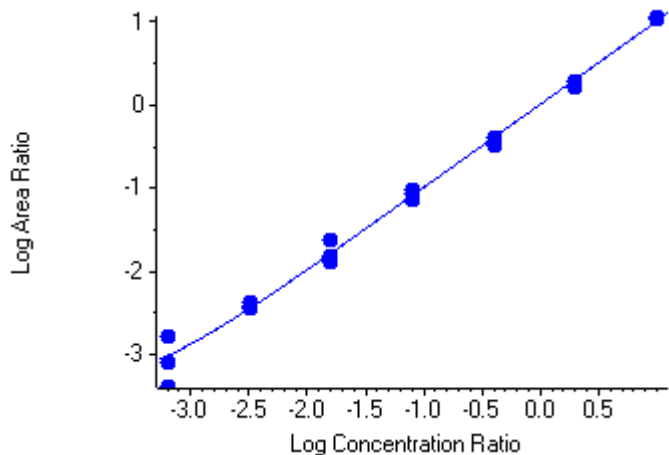
Analyte Name: Osteopontin.GDSLAYGLR.2y4.heavy

Calibration Equation: $y = 1.14243 x + 7.65854e-4$ ($r = 0.98849$) (weighting: $1 / x^2$)



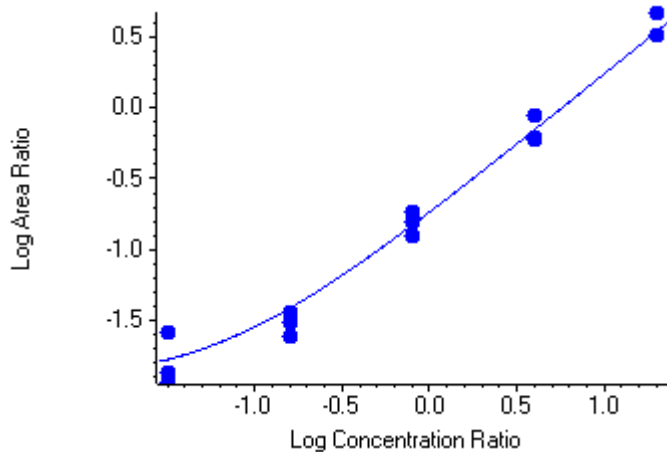
Analyte Name: Osteopontin.GDSLAYGLR.3y6.heavy

Calibration Equation: $y = 1.02837 x + 3.24011e-4$ ($r = 0.93214$) (weighting: $1 / x^2$)



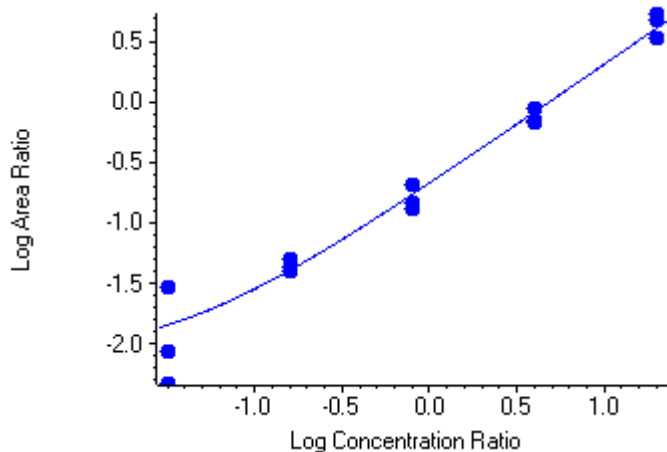
Analyte Name: Plectin.AGTLSITEFADMLSGNAGGFR.1y8.heavy

Calibration Equation: $y = 0.17272 x + 0.01127$ ($r = 0.82689$) (weighting: $1 / x^2$)



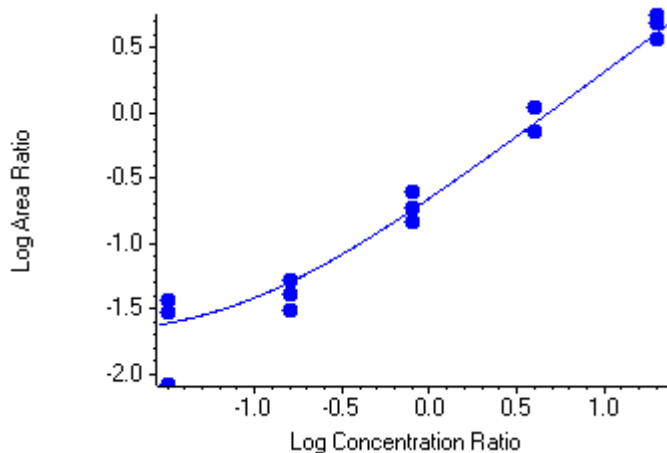
Analyte Name: Plectin.AGTLSITEFADMLSGNAGGFR.2y12.heavy

Calibration Equation: $y = 0.20985 x + 0.00777$ ($r = 0.74368$) (weighting: $1 / x^2$)



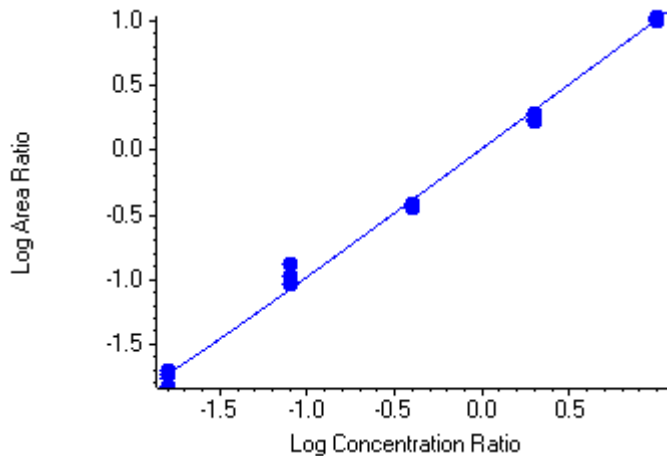
Analyte Name: Plectin.AGTLSITEFADMLSGNAGGFR.3y7.heavy

Calibration Equation: $y = 0.20410 x + 0.01792$ ($r = 0.69651$) (weighting: $1 / x^2$)



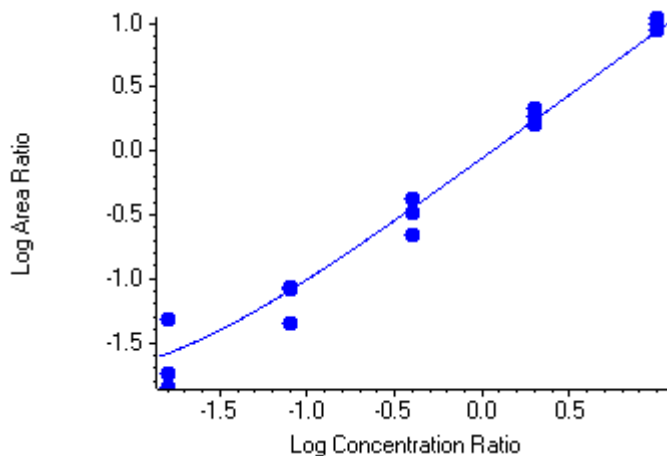
Analyte Name: TumorProteinD52.LGISSLQEFK.1y7.heavy

Calibration Equation: $y = 1.03168 x + 0.00217$ ($r = 0.97213$) (weighting: $1 / x^2$)



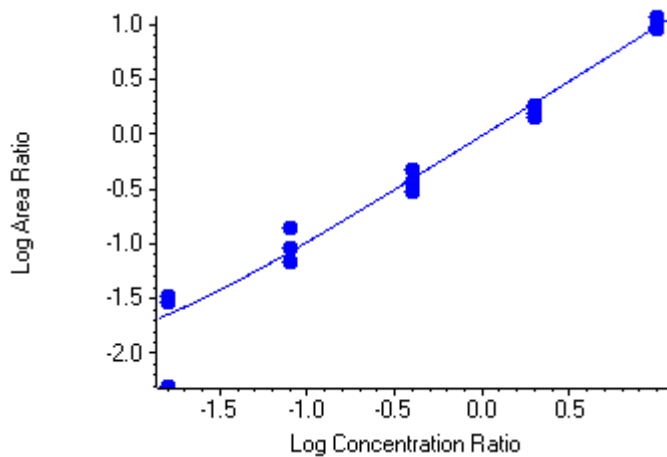
Analyte Name: TumorProteinD52.LGISSLQEFK.2y6.heavy

Calibration Equation: $y = 0.86584 x + 0.01214$ ($r = 0.84631$) (weighting: $1 / x^2$)



Analyte Name: TumorProteinD52.LGISSLQEFK.3y9.heavy

Calibration Equation: $y = 0.97222 x + 0.00721$ ($r = 0.89232$) (weighting: $1 / x^2$)



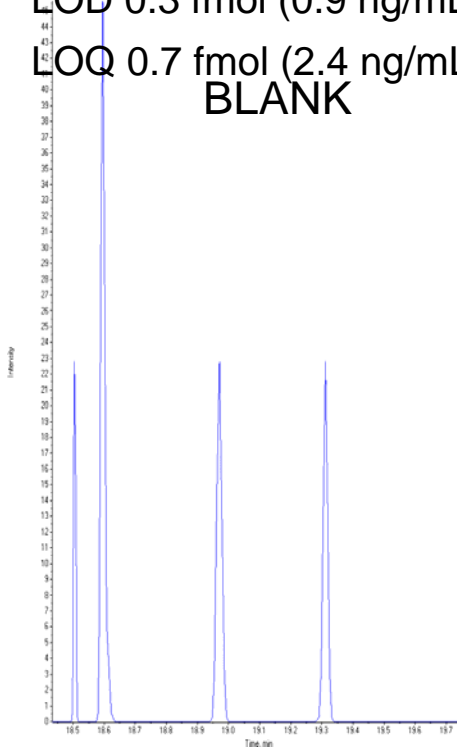
Supplemental Figure 3. Extracted ion MRM chromatograms are plotted for the most abundant transition for each peptide target at three levels around the estimated limit of detection and/or quantitation. The plots are useful for visual evaluation of the LODs/LOQs.

Calumenin.SFDQLTPEESK

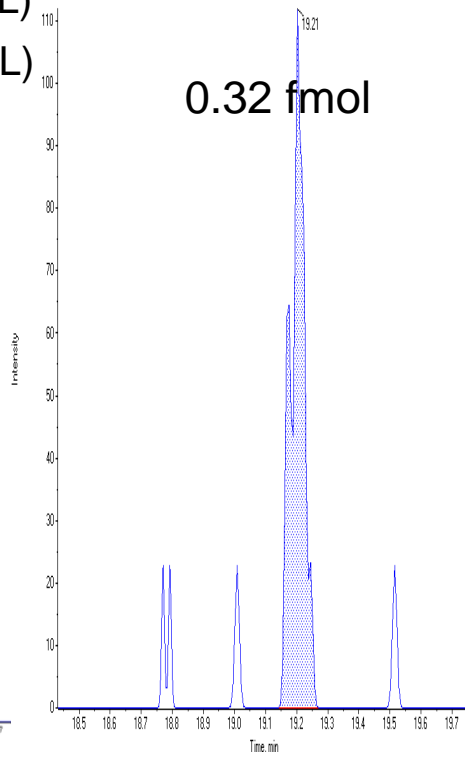
LOD 0.3 fmol (0.9 ng/mL)

LOQ 0.7 fmol (2.4 ng/mL)

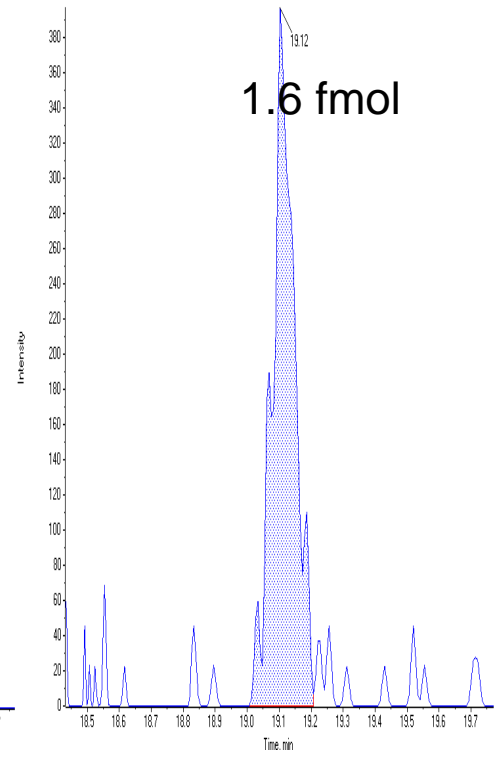
BLANK



0.32 fmol



1.6 fmol

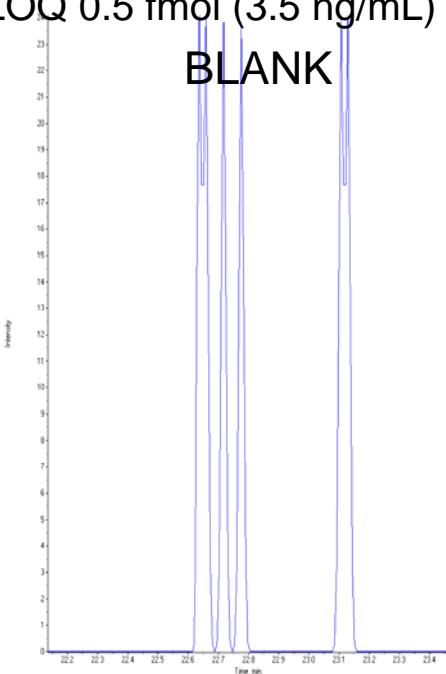


Disulfide isomerase.VEGFPTIYFAPSGDK

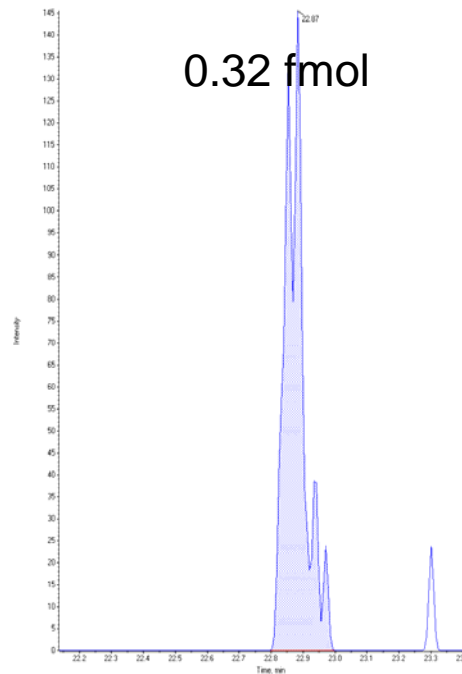
LOD 0.3 fmol (2.1 ng/mL)

LOQ 0.5 fmol (3.5 ng/mL)

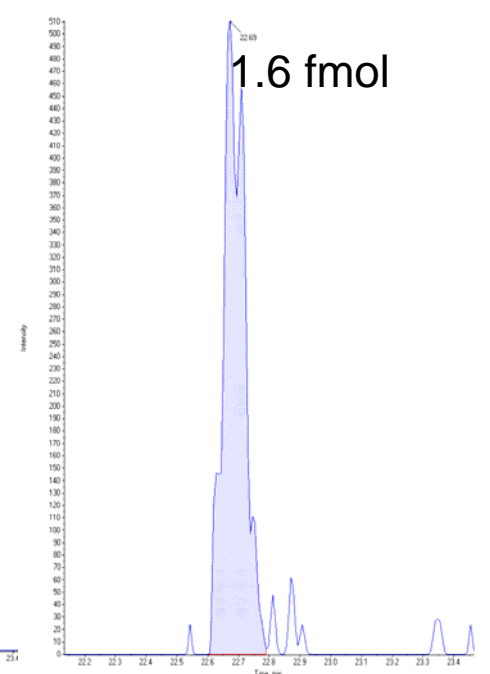
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0.32 fmol



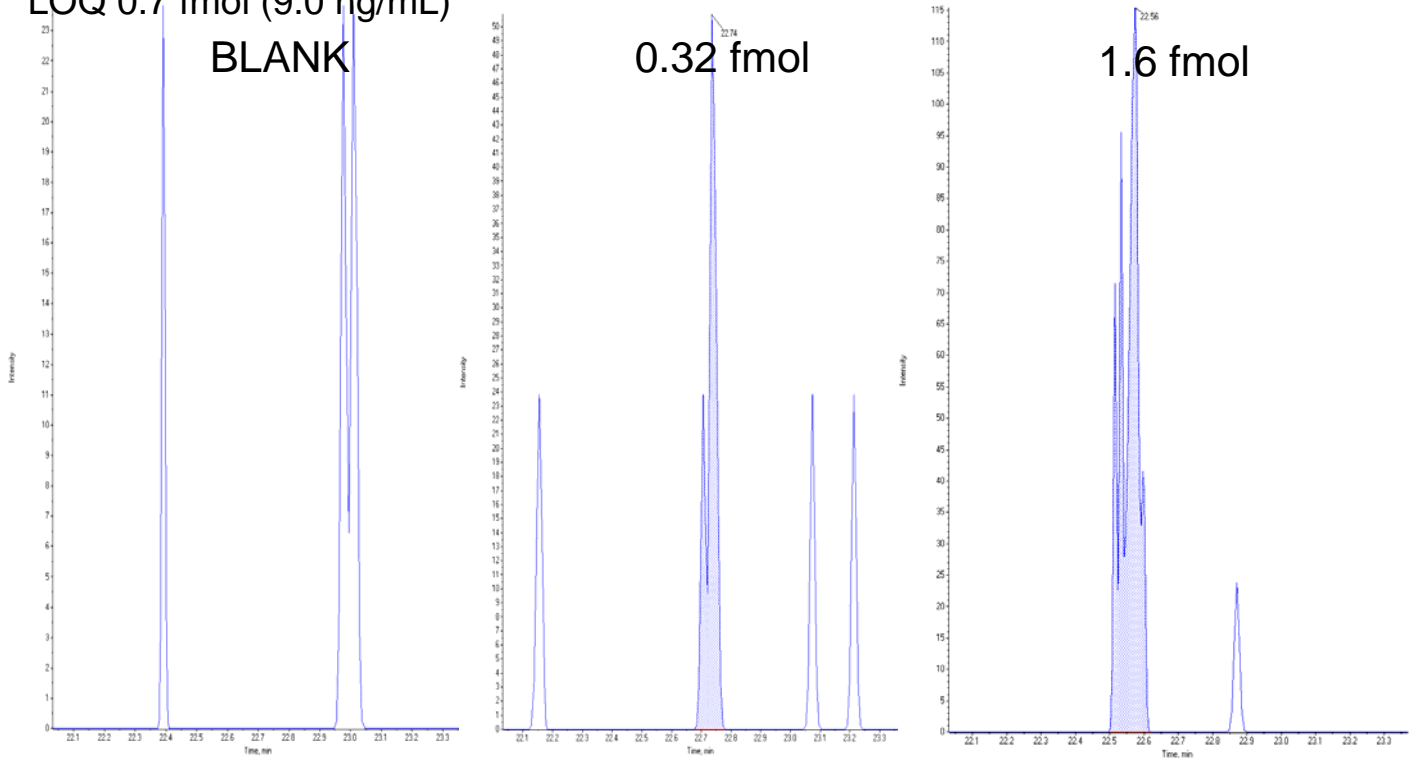
1.6 fmol



Fibulin-2.IGPAPAFAGDTISLTITK

LOD 0.3 fmol (3.9 ng/mL)

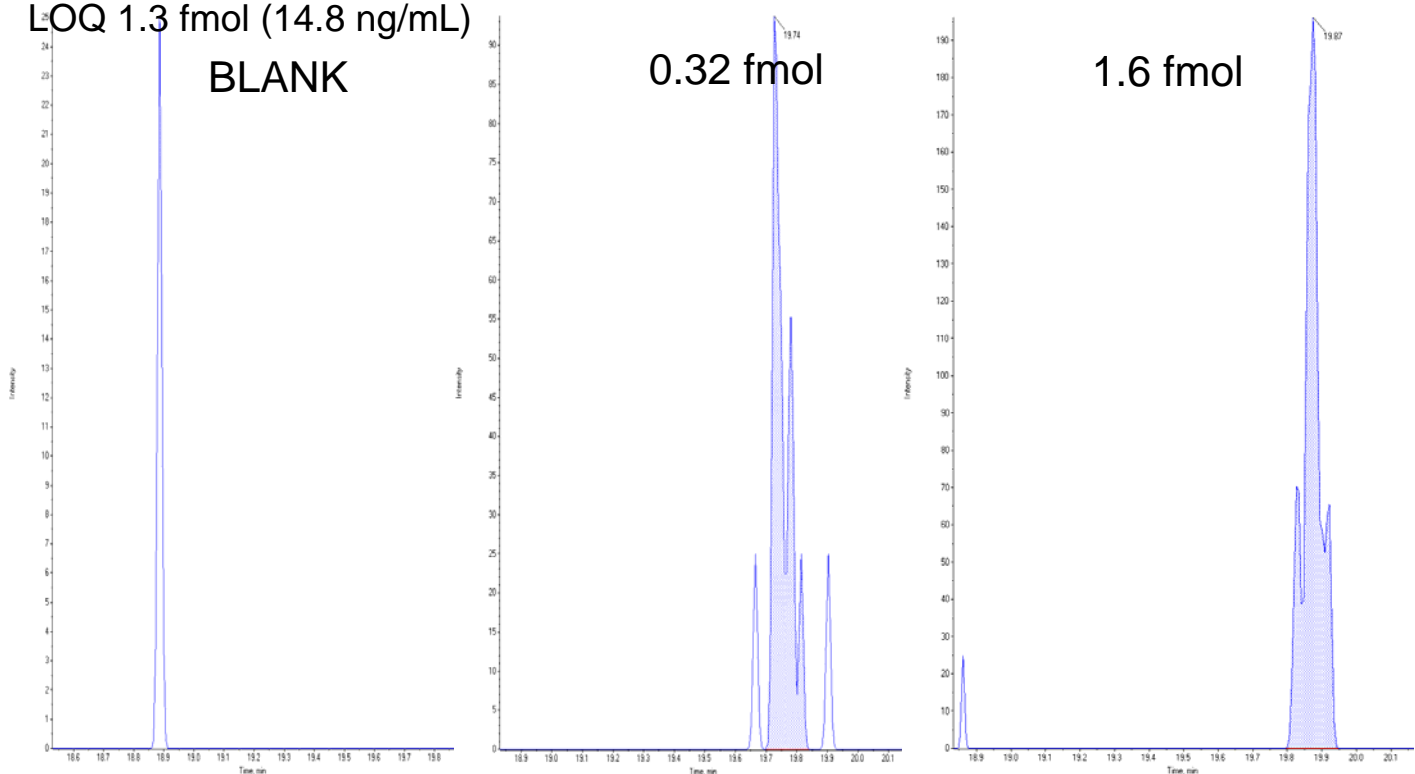
LOQ 0.7 fmol (9.0 ng/mL)



Hypoxia upregulated.LYQPEYQEVSTEEQR

LOD 0.5 fmol (5.9 ng/mL)

LOQ 1.3 fmol (14.8 ng/mL)

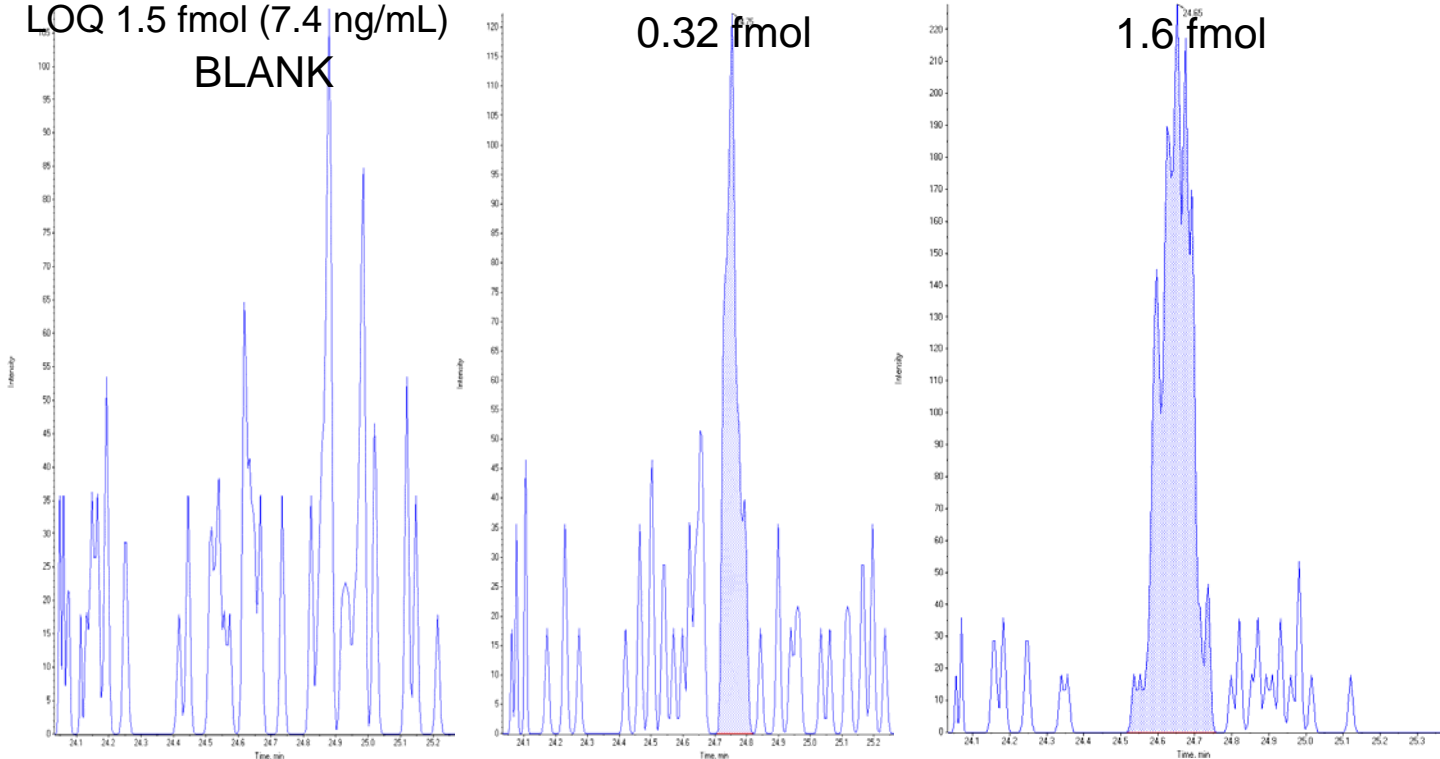


Legumain.DYTGEDVTPENFLAVLR

LOD 0.6 fmol (3.1 ng/mL)

LOQ 1.5 fmol (7.4 ng/mL)

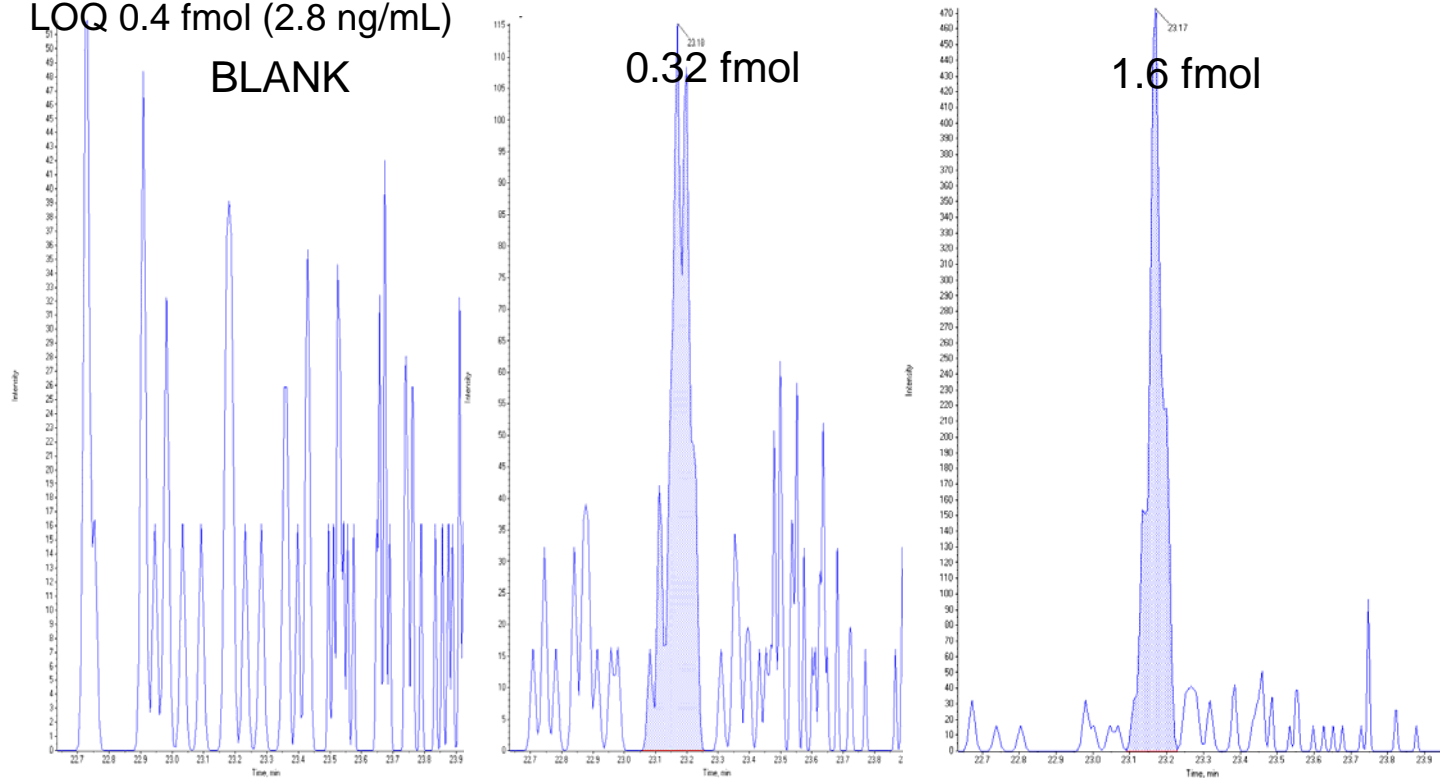
Supplemental Figure 3. Cont.



L-plastin.YTLNILEDIGGGQK

LOD 0.2 fmol (1.1 ng/mL)

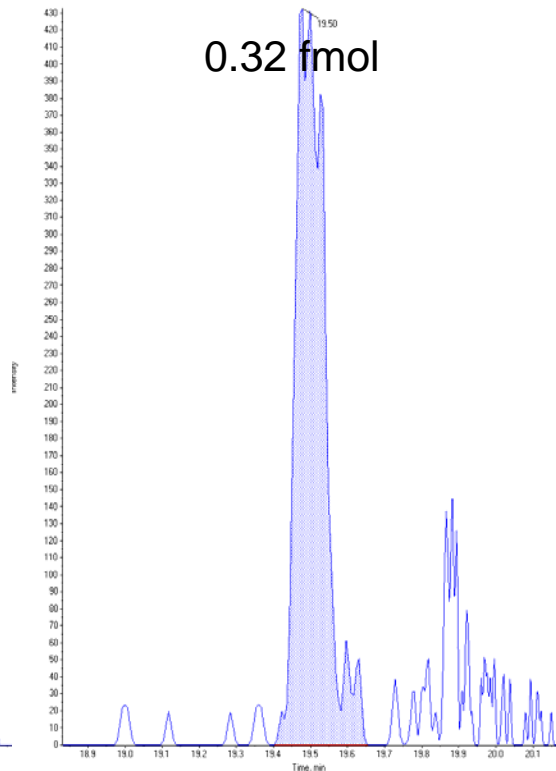
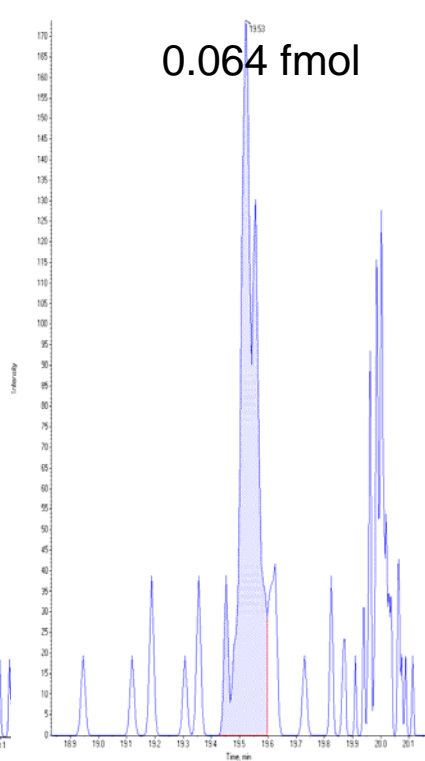
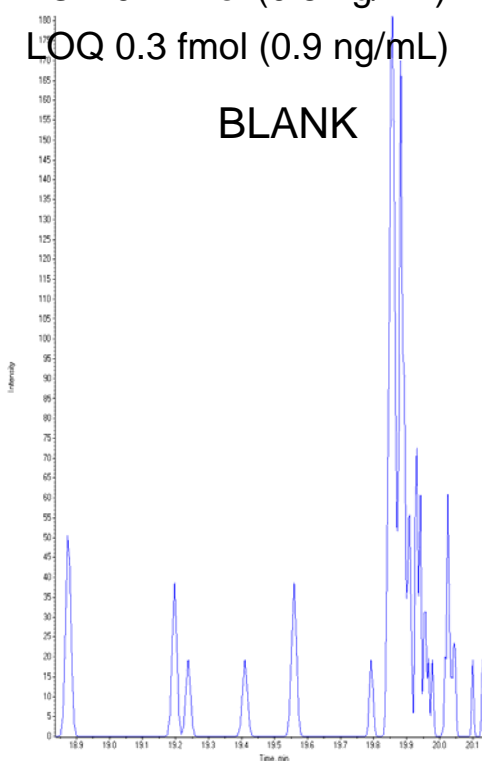
LOQ 0.4 fmol (2.8 ng/mL)



Osteopontin.GDSLAYGLR

LOD 0.1 fmol (0.3 ng/mL)

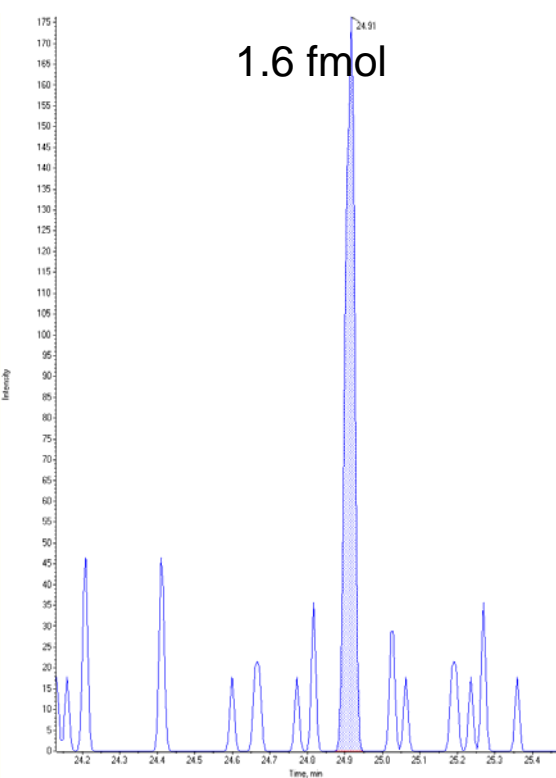
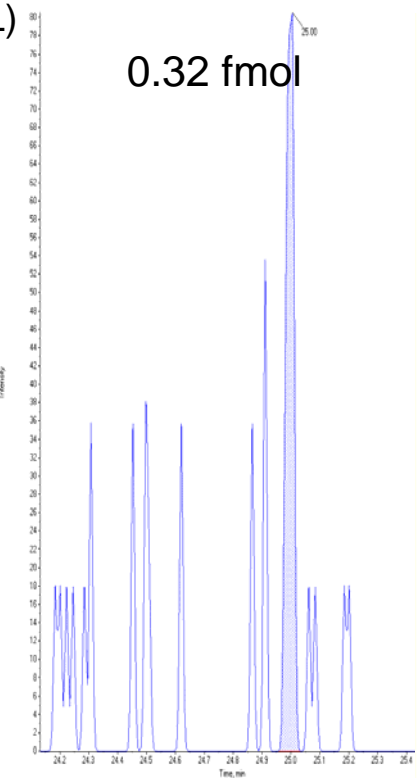
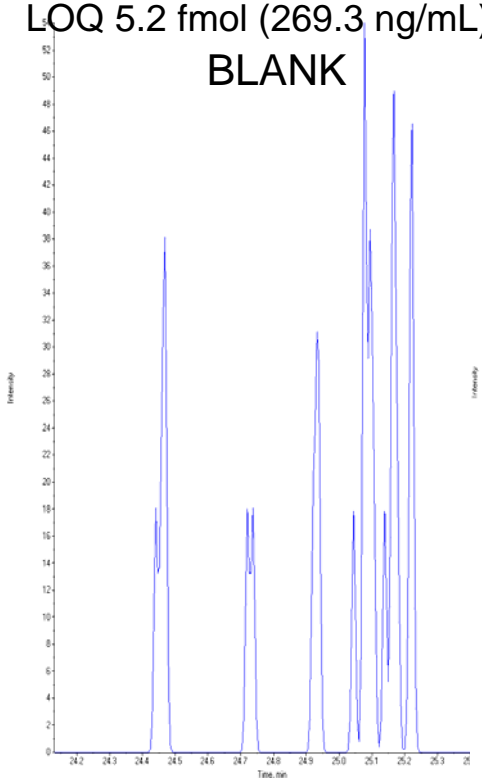
LOQ 0.3 fmol (0.9 ng/mL)



Plectin8.AGTLSITEFADMLSGNAGGFR

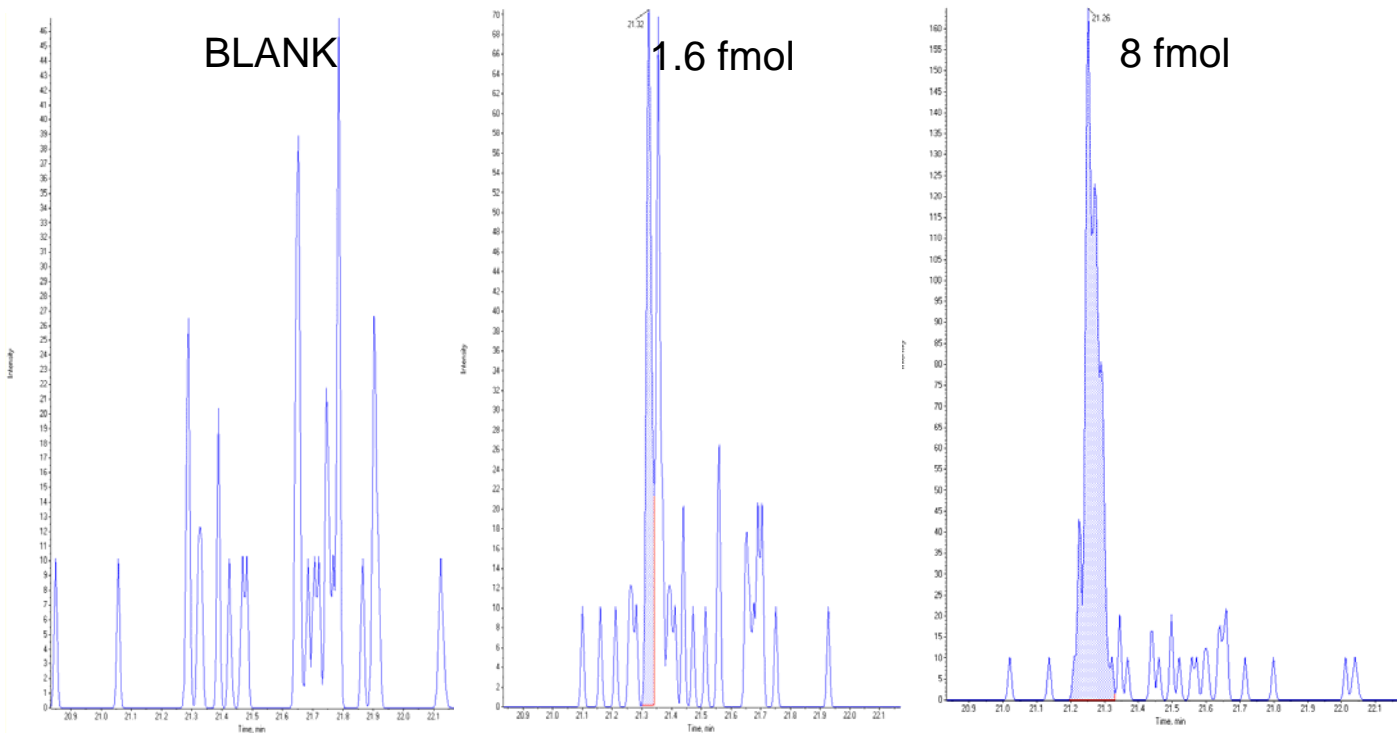
LOD 2.0 fmol (101.1 ng/mL)

LOQ 5.2 fmol (269.3 ng/mL)

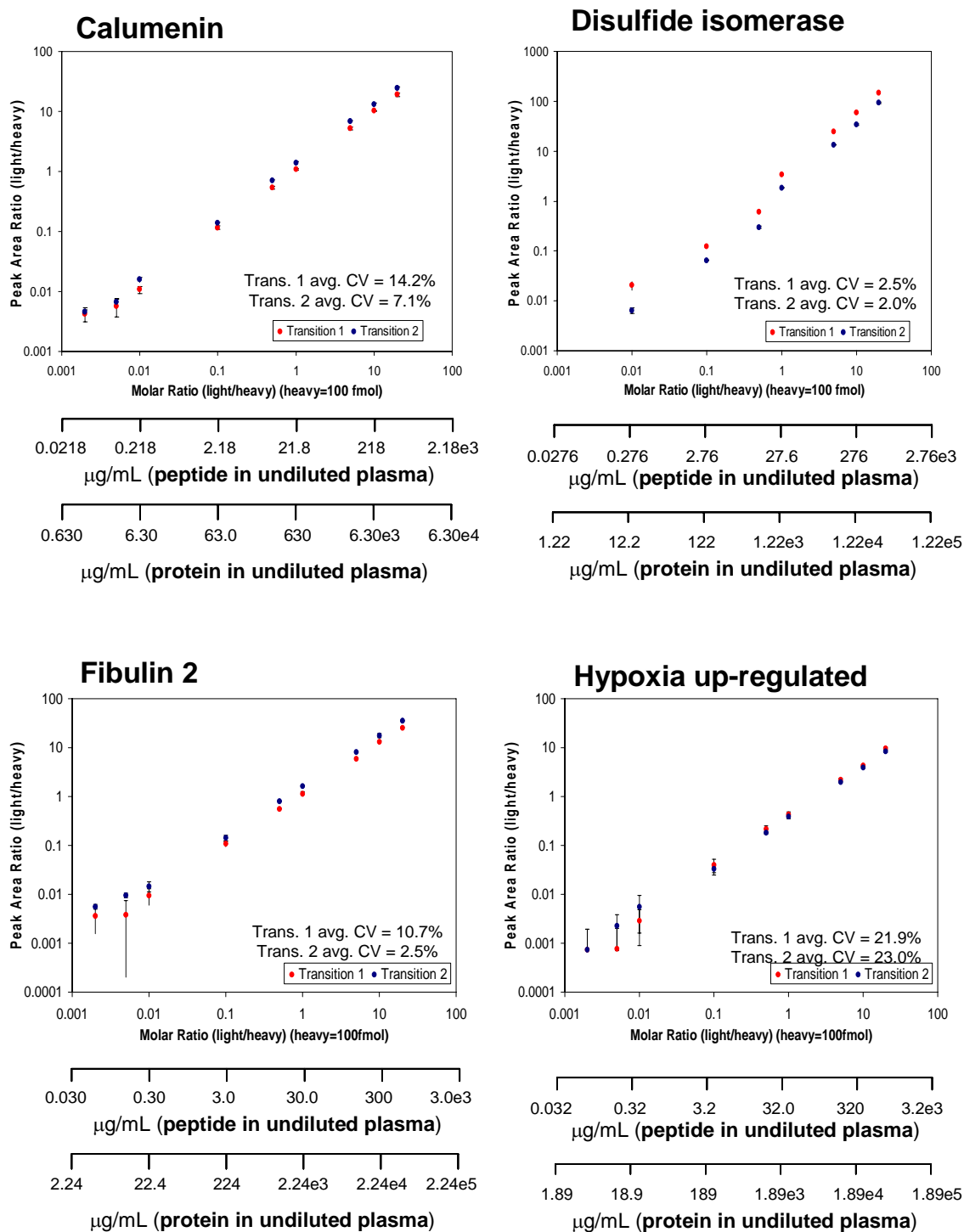


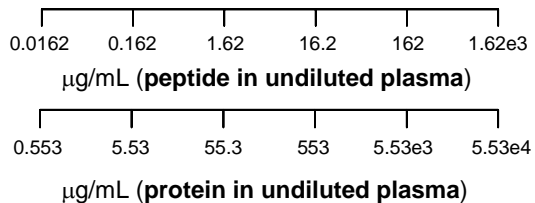
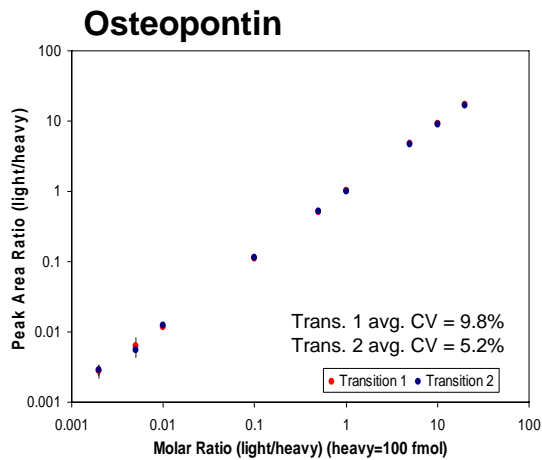
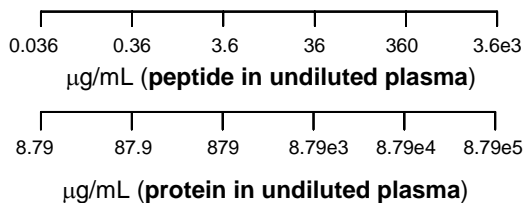
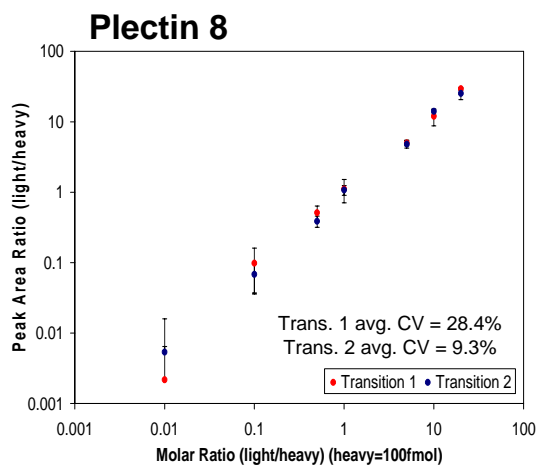
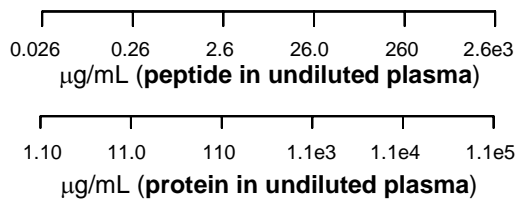
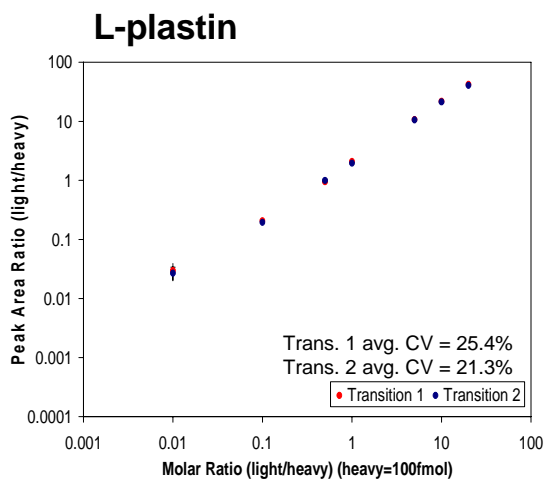
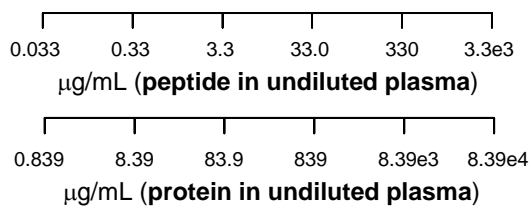
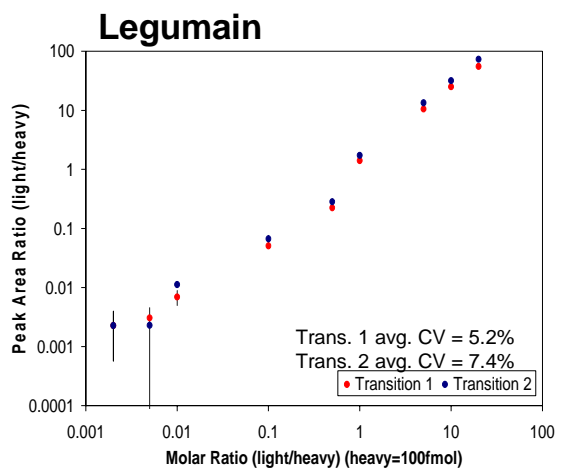
Tumor protein D52.LGISSLQEFK
LOD 3.7 fmol (7.5 ng/mL)
LOQ 10.4 fmol (20.8 ng/mL)

Supplemental Figure 4. Cont.



Supplemental Figure 4. Response curves without SISCAPA. Peptides were spiked into *undepleted* and unfractionated mouse plasma digest dilute 1:170. MRM measurements were performed on the spiked peptides after direct injection of 1 μ L of the diluted plasma.





Tumor protein D52

