Response Curve (Experiment 1)

Error category	Error message	Example case			Solution to the issue
Internal standard	Internal standard is set to	Internal			In Skyline, settings -> Peptide Settings -> Modifications ->
	be none.	Skyline File Name Standard Type	٩	Quality	Internal standard type, set Internal standard.
		MousePlasmaV2Exp1B3_refined.sky none The in	internal standard in one. Errors happen fo	the skyline file is set to	
Internal standard	Internal standard is	Internal			In Skyline, settings -> Peptide Settings -> Modifications ->
	incorrect according to the	Skyline File Name Standard Type	Qu	Jality	Internal standard type, set Internal standard to be the correct
	inferred internal standard.	MousePlasmaV2Exp1B3_refined.sky heavy Heavy, with Errors heavy.	ernal standard in the while the inferred in happen for all the pe	e skyline file is set to be nternal standard is light. peptides.	type.
Attribute	Essential attributes have				1. In Skyline, for the old template, View -> Document Grid ->
	missing values.	Skyline File Name Peptide ser	equence with rrors	Issue reason	Views -> Replicates, check the corresponding attributes; for the
				 Essential attribute(s) has (have) missing 	new template, View -> Document Grid -> Views -> Assay Curve
				ReplicateName; Replicate; SampleGroup.	Replicates , check the corresponding attributes.
		DDRpanel_052316_curves_05_QCTEST.sky DLSHIGDAVVISC[+	+57]AK[+114]DGVK	 Essential attribute(s) has (have) missing 	2. If the corresponding attributes are normal in step 1, in Skyline,
				values, including ISSpike or PeptideConcentrationIS.	View -> Targets -> By Name, in Targets panel, navigate to the
					peptide sequence, check the fragment ions from precursors of
					light and heavy isotopes, find the fragment ions with no data,
					and delete them.
Attribute	Essential attributes are	None			In Skyline, for the old template, View -> Document Grid -> Views
	annotated in unqualified				-> Replicates , check the data types of corresponding attributes;
	data type.				for the new template, View -> Document Grid -> Views -> Assay
					Curve Replicates, check the data types of corresponding
					attributes.
Internal standard	The annotation of the	Pentide			In Skyline, for the old template, View -> Document Grid -> Views
peptide	concentration of the	Skyline File Name sequence with errors	lss	sue reason	-> Replicates, check the attribute of IS Spike, then View ->
concentration	internal standard peptide		 The annotation of internal standard 	of the concentration of the od peptide has issues. Please	Document Grid -> Views -> Peptides , check the attribute of
	has issues.	MousePlasmaV2Exp1B3_refined.sky LPLPALFK	check the annota PeptideConcentra	ation of IS Spike or ationIS.	PeptideConcentrationIS; for the new template, View ->
					Document Grid -> Views -> Assay Peptides , check the attribute
					of Internal Standard Concentration.
Concentration	More than one	None			In Skyline, for the old template, View -> Document Grid -> Views
	concentration levels are				-> Replicates , check the attribute of Concentration; for the new

	needed.				template, View -> Document Grid -> Views -> Assay Curve Replicates, check the attribute of Analyte Concentration.
Light and Medium isotope	Both light and medium isotope labels are found in the peptide.	None			In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion to check its isotope label type.
Area values of heavy or light	More than one area values of heavy or light isotope	Skyline File Name	Peptide sequence with errors	Issue reason	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion and check the Peak Areas –
Isotope	exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel.	20160720_MousePlasmaExp1Batch5_refined.sky	ETPAASEAPSSAAK	 More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel. 	Replicate Comparison panel, more than one Peak Area value could be observed in one Replicate . Re-import the data related to the peptide into the current Skyline document to make sure there is one area value of heavy or light isotope kept.
Fragment ion	In response curve, no fragment ion with both heavy and light isotopes exists.	None			In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion (heavy or light isotope) and check the Peak Area value in Peak Areas – Replicate Comparison panel. Re-import the data related to the peptide into the current Skyline document to make sure the heavy and light isotope appear only one time for the fragment ions of the peptide.

Warning	Warning message	Example case	Solution to the issue
category			
Fragment ion	In response curve, the number of fragment ions with both heavy and light isotopes is less than 3.	In response curve, only two fragment ions 2.y4.1, 2.y5.1 (with both heavy and light isotopes) exist. Analyte: Q96B36.LNT[+80]SDFQK	Check the response curve graph to make further decisions.
Bad linear regression fit	The coefficient of variance of slopes of the fragment ions is larger than 0.5.	Analyte: OS5819.VYPLINR	Check the slopes of the fragment ions in the response curve graph to make further decisions.

Bad linear regression fitting	The fit of the linear regression model is poor due to R ² < 0.5 or p > 0.05 in the significance test for linear regression.	Analyte: Q92851.HEDILSILTAVNDDVSR	Check the response curve graph to make further decisions.
Bad linear regression fitting	The exponent of the fitted power function for the fragment ion data is not between 0.75 and 1.25	None	Check the response curve graph to make further decisions.
High variance	Not all of the transition ratios of the fragment ion are within 30% of the mean when checking the samples above the LLOQ	None	Check the response curve graph to make further decisions.

Repeatability (Experiment 2)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to	None	In Skyline, settings -> Peptide Settings -> Modifications ->
	be none.		Internal standard type, set Internal standard.
Internal standard	Internal standard is	Internal	In Skyline, settings -> Peptide Settings -> Modifications ->
	incorrect according to the	Skyline File Name Standard Quality Type	Internal standard type, set Internal standard to be the correct
	inferred internal standard.	TCGA glyco 26 peps_repeatability.sky light The internal standard in the skyline file is set to be all the peptides.	type.
Attribute	Essential attributes have		In Skyline, for the old template, View -> Document Grid -> Views
	missing values.	Skyline File Name Peptide sequence with errors Issue re	-> Replicates , check the corresponding attributes; for the new
		• Essenti attribu (have)	template, View -> Document Grid -> Views -> Assay
		DDRpanel_052316_repeatability_03_QCTEST.sky LSLEFPSGYPYNAPTVK, precursor charge: 2 values, cludi Replica Concent SampleG	Repeatability Replicates, check the corresponding attributes.
Attribute	Essential attributes are	None	In Skyline, for the old template, View -> Document Grid -> Views
	using uncontrolled terms.		-> Replicates, check the corresponding attributes; for the new
			template, View -> Document Grid -> Views -> Assay
			Repeatability Replicates , check the corresponding attributes.
Attribute	Essential attributes are	None	In Skyline, for the old template, View -> Document Grid -> Views
	annotated in unqualified		-> Replicates, check the data type of the corresponding
	data type.		attributes; for the new template, View -> Document Grid ->
			Views -> Assay Repeatability Replicates, check the data type of
			the corresponding attributes.
Light and	Both light and medium	None	In Skyline, in the Targets panel, navigate to the peptide with
Medium isotope	isotope labels are found in		errors, click each fragment ion to check its isotope label type.
	the peptide with a specific		
	charge.		

Area values of	The number of light		Pontido		In Skyline, for the old template, View -> Document Grid ->
heavy or light	isotopes or heavy isotopes	Skyline File Name	sequence with errors	issue reason	Views -> Replicates, check the values of the attribute of
Isotope	is not one due to wrongly			 For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in 	Replicate for the problematic replicate names, for example,
	annotated values in			<pre>attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For b3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y3 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03, For y6 (1+): 2 heavy isotopes</pre>	correct the values of the attribute of Replicate for the replicate
	attributes.				name of Day4_Std3_R01 and Day4_Std3_R03. In Skyline, for the
		MousePlasmaExp2B3_Refined.sky precursor charg	precursor charge: 2		old template, View -> Document Grid -> Views -> Replicates,
					check the values of the attribute of Replicate for the
				2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Dav4_Std3_R03).	problematic replicate names; for the new template, View ->
					Document Grid -> Views -> Assay Repeatability Replicates,
					check the values of the attribute of Replicate Number for the
					problematic replicate names.

Warning	Warning message	Example case Solution to the issue
category		
Fragment ion	In repeatability graph, the	In the repeatability graph below, the number of fragment ions Check the repeatability graph to make further decisions.
	number of fragment ions	is 1 < 3; the only fragment ion is: y7 (1+).
	with both heavy and light	
	isotopes is less than 3.	straujuoLivioirevdevsek y7 (1+) sum olinis
		ed (are
		a i i i j 1 2 3 4 5 Time (day) Time (day) Time (day)
	la na sata bilita ana da fan	For forework in (44.(2)), there are no with an also 4. Check the constant life much to make for the devicing
Missing points	In repeatability graph, for	For fragment ion y14 (2+), there are no points on day 4. Check the repeatability graph to make further decisions.
	the fragment ion, there is	»
	no point on at least one	
	uay.	origi origi भाष
		G G G G
		ed (a
		1 2 3 4 5 Time (day)
Missing points	In repeatability graph,	None Check the repeatability graph to make further decisions.
	fragment ion doesn't have	
	three concentrations: Hi,	
	Med, and Low on a specific	

	day.		
Missing points	In repeatability graph,	For fragment ion y5 (1+), there are less than 3 replicates in	Check the repeatability graph to make further decisions.
	fragment ion has less than	concentration(s) Hi, Lo, Med on day 1.	
	3 replicates for a specific	GLDARPEVTR v5 (1+)	
	concentration on the specific day.	Weastrice (area ratio) [00-scale]	
Bad distribution	In repeatability graph, for	None	Check the repeatability graph to make further decisions.
of points	the fragment ion, the intra-		
	assay, inter-assay or total		
	coefficient of variance is		
	larger than the threshold of		
	0.2.		
High variance	Not of all the transition	None.	Check the repeatability graph to make further decisions.
	ratios of the fragment ion in		
	the specific concentration		
	are within 30% of the		
	mean.		



Selectivity (Experiment 3)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to	Internal	In Skyline, settings -> Peptide Settings -> Modifications ->
	be none.	Skyline File Name Standard Quality Type	Internal standard type, set Internal standard.
		The internal standard in the skyline file is set to be none. Please set it to be heavy. Errors happen for all the mortides	
Internal standard	Internal standard is		In Skyline, settings -> Peptide Settings -> Modifications ->
	incorrect.	Internal Skyline File Name Standard Quality	Internal standard type, set Internal standard to be the correct
		Type The internal standard in the skuline file is set to be	type.
		Batch1_Exp3_20160519_M1.sky heavy heavy, while the infered internal standard is light. Errors happen for all the peptides.	
Attribute	Essential attributes have	None	In Skyline, View -> Document Grid -> Views -> Assay Selectivity
	missing values.		Replicates , check the corresponding attributes.
Attribute	Essential attributes are	None	In Skyline, View -> Document Grid -> Views -> Assay Selectivity
	annotated in unqualified		Replicates , check the data types of corresponding attributes.
	data type.		
Light and	Both light and medium	None	In Skyline, in the Targets panel, navigate to the peptide with
Medium isotope	isotope labels are found in		errors, click each fragment ion to check its isotope label type.
	the peptide with a specific		
	charge.		
Concentration	More than one	None	In Skyline, View -> Document Grid -> Views -> Assay Selectivity
	concentration levels are		Replicates , check the attribute of Analyte Concentration.
	needed.		
Area values of	The number of light		In Skyline, View -> Document Grid -> Views -> Assay Selectivity
heavy or light	isotopes or heavy isotopes	Skyline File Name sequence with Issue reason errors	Replicates, check the values of the attribute of Replicate
Isotope	is not one due to wrongly	 Tor yr (1): 2 Reavy isotops: 2 light isotops do to wrady? another wrady another value in attributes: regulates, analyte construction or sample groups, for yr (1): 2 heavy isotops: 2 light isotops do to mongly another value in attributes: regulates; provide the sample another value in attributes; regulates; provide the sample another value in attributes; provide the sample and the sample and the sample another value in attributes; provide the sample and the samp	Number, Analyte Concentration and Exp3 SampleGroup.
	annotated values in	11-20_16-51-53.sky.zip precursion carping progr. for y9 (1): 2 havy interpret 2 [jight interpret and progr. for y9 (1): 2 havy interpret 2 [jight interpret and progr. for y9 (2): 2 havy interpret 2 [jight interpret an	
	attributes.		

Warning	Warning message	Example case	Solution to the issue
category			
Fragment ion	The number of fragment	None	Check the selectivity graph to make further decisions.
	ions is less than 3.		
Sample group	The number of sample	None	Check the selectivity graph to make further decisions.
	group is less than 6.		
Bad linear	The coefficient of variance	None	Check the selectivity graph to make further decisions.
regression fit	of slopes of the fragment		
	ion is larger than 0.5.		
Bad linear	The fit of the linear	None	Check the selectivity graph to make further decisions.
regression fitting	regression model is poor		
	due to $R^2 < 0.5$ for some		
	fragment ions in the		
	regression		
Bad linear	The RMSE of intercepts at		Check the area values for the fragment ion v6 (1+) in Skyline.
regression fitting	y axis of the fit lines is	For fragment ion of y6 (1+), the RMSE of intercepts at y axis of	3 7 7 7
-	, larger than 1.0.	is 1.008 > 1.	
		VLILAYAVC[+57]R	
		y6 (1+)	
		8-	
		Tell line	
		6 • • • • • • • • • • • • • • • • • • •	
		ponsee	
		Spike-in level	

High variance	Not all of the area ratios of	Nene	Check the area values for the fragment ion in Skyline.
	the fragment ion in the	None	
	non-blank spike level are		
	within 30% of the mean.		

Stability (Experiment 4)

Error category	Error message	Example case				Solution to the issue
Internal standard	Internal standard is set to		Internal			In Skyline, settings -> Peptide Settings -> Modifications ->
	be none.	Skyline File Name	Standard Type	d	Quality	Internal standard type, set Internal standard.
		CPTAC_TemplateDoc_Exp4_Stability_DRAFT02_2020- 01-06_16-35-35.sky.zip	none	The internal standar none. Please set it the peptides.	d in the skyline file is set to be to be heavy. Errors happen for all	
Attribute	Essential attributes have			Peptide		1. In Skyline, View -> Document Grid -> Views -> Assay Stability
	missing values.	Skyline File Name		sequence with errors	Issue reason	Replicates , check the corresponding attributes.
		CPTAC_TemplateDoc_Exp4_Stability_DRAF 01-09_10-45-35.sky.zip	T02_2020-	LDQPGNLPGSNR, precursor charge: 2	 Essential attribute(s) has (have) missing values, including ReplicateNumber. 	
Attribute	Essential attributes are	None				In Skyline, View -> Document Grid -> Views -> Assay Stability
	annotated in unqualified					Replicates , check the data types of corresponding attributes.
	data type.					
Attribute	Incorrect annotations in	None				In Skyline, View -> Document Grid -> Views -> Assay Stability
	the attribute of Exp4					Replicates , check the corresponding attributes.
	Sample Group or Freeze					
	Thaw Cycles.					
Light and	Both light and medium	None				In Skyline, in the Targets panel, navigate to the peptide with
Medium isotope	isotope labels are found in					errors, click each fragment ion to check its isotope label type.
	the peptide with a specific					
	charge.					
Area values of	The number of light	P	eptide			In Skyline, View -> Document Grid -> Views -> Assay Stability
heavy or light	isotopes or heavy isotopes	Skyline File Name sequ e	ence with errors		Issue reason	Replicates, check the values of the attribute of Replicate
Isotope	is not one due to wrongly			 For y5 (1+): 2 heavy is annotated values in att group. For y6 (1+): 2 h wrongly annotated value 	sotopes 2 light isotopes due to wrongly rributes: replicate number, time or sample neavy isotopes 2 light isotopes due to rs in attributes: replicate number, time or	Number, Time, Time Units and Exp4 SampleGroup.
	annotated values in	CPTAC_TemplateDoc_Exp4_Stability_DRAFT02_2020- 01-16_10-45-35.sky.zip LDQPGM	ILPGSNR, rson e: 2	sample group. For y8 (1 to wrongly annotated va or sample group. For y9 due to wrongly annotate	(+): 2 heavy isotopes 2 light isotopes due slues in attributes: replicate number, time 0 (1+): 2 heavy isotopes 2 light isotopes ad values in attributes: replicate number.	
	attributes.			time or sample group. F isotopes due to wrongly number, time or sample	or y9 (2+): 2 heavy isotopes 2 light / annotated values in attributes: replicate group.	

Warning	Warning message	Example case	Solution to the issue
category			
Attribute	Attributes of Time or Time	None	In Skyline, View -> Document Grid -> Views -> Assay Stability
	Units have missing values.		Replicates, check the corresponding attributes.
Sample storage conditions and times	The number of sample storage conditions and times is less than 6.	GTFC[+57]SFDTPDDSIR Y10 (1) P-value from ANOVA: 0.94	Check the stability graph to make further decisions.
Replicate Number	The number of replicate is less than 2.	None	Check the stability graph to make further decisions.
Significant changes observed	The ANOVA p value is less than 0.05.	VGFLPSAGK y7 (1+) P-value from ANOVA: 0.0046 Contilion	Check the stability graph to make further decisions.
Bad distribution of points	In stability table, for the fragment ion under the	None	Check the stability table to make further decisions.

	storage condition, the		
	intra-assay of variance is		
	larger than the threshold of		
	0.2.		
Bad distribution	In stability table, for the	None	Check the stability table to make further decisions.
of points	fragment ion, according to	None	
	time zero analysis, the		
	RMSE(s) is larger than the		
	threshold of 0.2.		

Endogenous (Experiment 5)

Error category	Error message	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	Skyline File Name Internal Standard Type Quality CPTAC_TemplateDoc_Exp5_Reproducibility_DRAFT01_2020* 03-26_09-58-55.sky.zip The internal standard in the skyline file is set to be none. Errors happen for all the peptides.	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type, set Internal standard.
Attribute	Essential attributes have missing values.	Skyline File Name Peptide sequence with errors Issue reason CPTAC_TemplateDoc_Exp5_Reproducibility_DRAFT01_2020- 03-25_09-58-55.sky.zip LDQPONLPGSNR, precursor charge: 2 • Essential attribute(s) has (have) missing values, including Day. • Essential attribute(s) has (have) missing • Essential attribute(s) has (have) missing	In Skyline, View -> Document Grid -> Views -> Assay Endogenous Replicates, check the corresponding attributes.
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, View -> Document Grid -> Views -> Assay Endogenous Replicates, check the data types of corresponding attributes.
Light and Medium isotope	Both light and medium isotope labels are found in the peptide with a specific charge.	None	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion to check its isotope label type.
Area values of heavy or light Isotope	The number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes.	None	In Skyline, View -> Document Grid -> Views -> Assay Endogenous Replicates, check the values of the attribute of Replicate Number, Day and SampleGroup.

Warning	Warning message	Example case	Solution to the issue
category			
Fragment ion	In reproducible detection	None	Check the reproducible detection of endogenous analyte graph
	of endogenous analyte		to make further decisions.
	graph, the number of		
	fragment ions with both		
	heavy and light isotopes is		
	less than 3.		
Missing points	In reproducible detection	None	Check the reproducible detection of endogenous analyte graph
	of endogenous analyte		to make further decisions.
	graph, for the fragment ion,		
	there is no point on at least		
	one day.		
Missing points	In reproducible detection	None	Check the reproducible detection of endogenous analyte graph
	of endogenous analyte		to make further decisions.
	graph, fragment ion has		
	less than 3 replicates on the		
	specific day.		