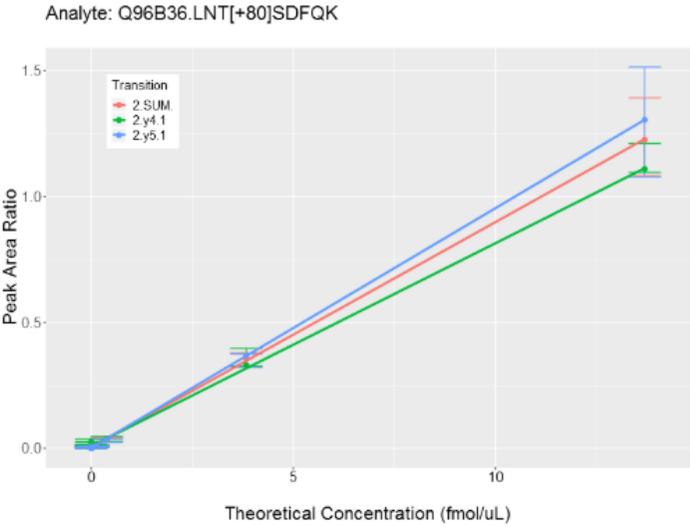
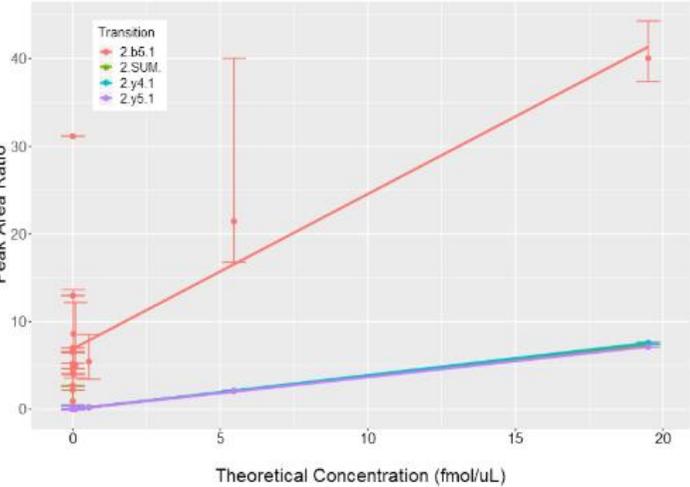


Response Curve (Experiment 1)

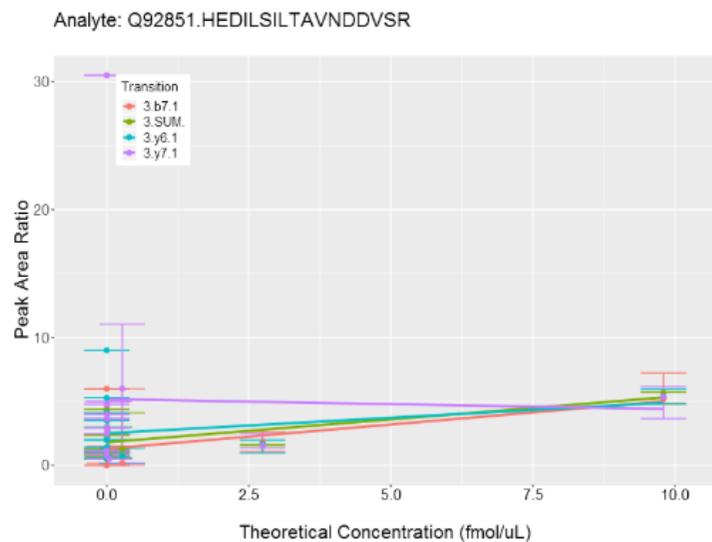
Error category	Error message	Example case	Solution to the issue						
Internal standard	Internal standard is set to be none.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Internal Standard Type</th> <th>Quality</th> </tr> </thead> <tbody> <tr> <td>MousePlasmaV2Exp1B3_refined.sky</td> <td>none</td> <td>The internal standard in the skyline file is set to be none. Errors happen for all the peptides.</td> </tr> </tbody> </table>	Skyline File Name	Internal Standard Type	Quality	MousePlasmaV2Exp1B3_refined.sky	none	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.
Skyline File Name	Internal Standard Type	Quality							
MousePlasmaV2Exp1B3_refined.sky	none	The internal standard in the skyline file is set to be none. Errors happen for all the peptides.							
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Internal Standard Type</th> <th>Quality</th> </tr> </thead> <tbody> <tr> <td>MousePlasmaV2Exp1B3_refined.sky</td> <td>heavy</td> <td>The internal standard in the skyline file is set to be heavy, while the inferred internal standard is light. Errors happen for all the peptides.</td> </tr> </tbody> </table>	Skyline File Name	Internal Standard Type	Quality	MousePlasmaV2Exp1B3_refined.sky	heavy	The internal standard in the skyline file is set to be heavy, while the inferred internal standard is light. Errors happen for all the peptides.	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type , set Internal standard to be the correct type.
Skyline File Name	Internal Standard Type	Quality							
MousePlasmaV2Exp1B3_refined.sky	heavy	The internal standard in the skyline file is set to be heavy, while the inferred internal standard is light. Errors happen for all the peptides.							
Attribute	Essential attributes have missing values.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Peptide sequence with errors</th> <th>Issue reason</th> </tr> </thead> <tbody> <tr> <td>DDRpanel_052316_curves_05_QCTEST.sky</td> <td>DLSHIGDAVVISC[+57]JAK[+114]DGVK</td> <td> <ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including ReplicateName; Replicate; SampleGroup. Essential attribute(s) has (have) missing values, including ISSpike or PeptideConcentrationIS. </td> </tr> </tbody> </table>	Skyline File Name	Peptide sequence with errors	Issue reason	DDRpanel_052316_curves_05_QCTEST.sky	DLSHIGDAVVISC[+57]JAK[+114]DGVK	<ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including ReplicateName; Replicate; SampleGroup. Essential attribute(s) has (have) missing values, including ISSpike or PeptideConcentrationIS. 	<ol style="list-style-type: none"> In Skyline, for the old template, View -> Document Grid -> Views -> Replicates, check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Curve Replicates, check the corresponding attributes. If the corresponding attributes are normal in step 1, in Skyline, 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.
Skyline File Name	Peptide sequence with errors	Issue reason							
DDRpanel_052316_curves_05_QCTEST.sky	DLSHIGDAVVISC[+57]JAK[+114]DGVK	<ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including ReplicateName; Replicate; SampleGroup. Essential attribute(s) has (have) missing values, including ISSpike or PeptideConcentrationIS. 							
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the data types of corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Curve Replicates , check the data types of corresponding attributes.						
Internal standard peptide concentration	The annotation of the concentration of the internal standard peptide has issues.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Peptide sequence with errors</th> <th>Issue reason</th> </tr> </thead> <tbody> <tr> <td>MousePlasmaV2Exp1B3_refined.sky</td> <td>LPLPALFK</td> <td> <ul style="list-style-type: none"> The annotation of the concentration of the internal standard peptide has issues. Please check the annotation of IS Spike or PeptideConcentrationIS. </td> </tr> </tbody> </table>	Skyline File Name	Peptide sequence with errors	Issue reason	MousePlasmaV2Exp1B3_refined.sky	LPLPALFK	<ul style="list-style-type: none"> The annotation of the concentration of the internal standard peptide has issues. Please check the annotation of IS Spike or PeptideConcentrationIS. 	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the attribute of IS Spike, then View -> Document Grid -> Views -> Peptides , check the attribute of PeptideConcentrationIS; for the new template, View -> Document Grid -> Views -> Assay Peptides , check the attribute of Internal Standard Concentration.
Skyline File Name	Peptide sequence with errors	Issue reason							
MousePlasmaV2Exp1B3_refined.sky	LPLPALFK	<ul style="list-style-type: none"> The annotation of the concentration of the internal standard peptide has issues. Please check the annotation of IS Spike or PeptideConcentrationIS. 							
Concentration	More than one concentration levels are	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the attribute of Concentration; for the new						

	needed.		template, View -> Document Grid -> Views -> Assay Curve Replicates , check the attribute of Analyte Concentration.						
Area values of heavy or light Isotope	More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Peptide sequence with errors</th> <th>Issue reason</th> </tr> </thead> <tbody> <tr> <td>20160720_MousePlasmaExp18batch5_refined.sky</td> <td>ETPAASEAPSSAAK</td> <td> <ul style="list-style-type: none"> More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel. </td> </tr> </tbody> </table>	Skyline File Name	Peptide sequence with errors	Issue reason	20160720_MousePlasmaExp18batch5_refined.sky	ETPAASEAPSSAAK	<ul style="list-style-type: none"> More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel. 	In Skyline, in the Targets panel, navigate to the peptide with errors, click each fragment ion and check the Peak Areas – 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.
Skyline File Name	Peptide sequence with errors	Issue reason							
20160720_MousePlasmaExp18batch5_refined.sky	ETPAASEAPSSAAK	<ul style="list-style-type: none"> More than one area values of heavy or light isotope exist for the combination of protein, peptidemodifiedsequence, precursorcharge, productcharge, fragmentation, replicate, concentration, samplegroup, isspike and isotopelabel. 							
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 category	Warning message	Example case	Solution to the issue
Fragment ion	In response curve, the number of fragment ions with both heavy and light isotopes is less than 3.	<p>In response curve, only two fragment ions 2.y4.1, 2.y5.1 (with both heavy and light isotopes) exist.</p> 	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.	<p>Analyte: O95819.VYPLINR</p> 	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.



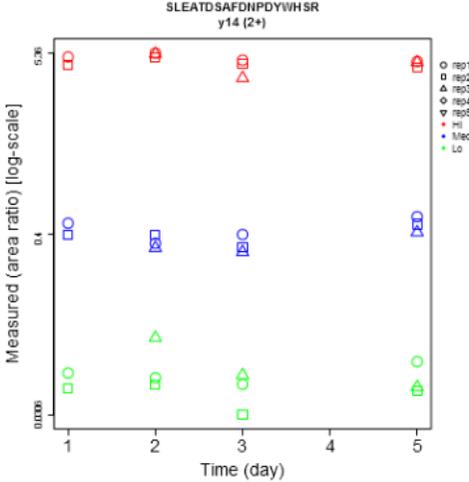
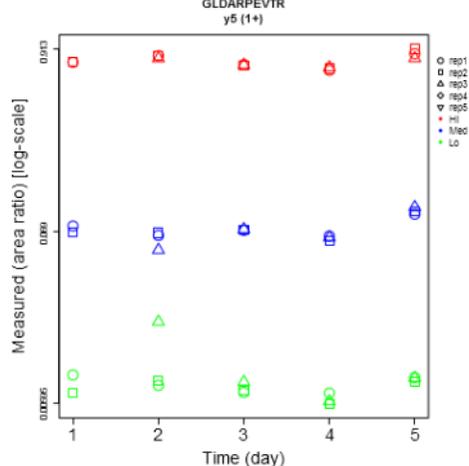
Check the response curve graph to make further decisions.

Repeatability (Experiment 2)

Error category	Error message	Example case	Solution to the issue						
Internal standard	Internal standard is set to be none.	None	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type , set Internal standard.						
Internal standard	Internal standard is incorrect according to the inferred internal standard.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Internal Standard Type</th> <th>Quality</th> </tr> </thead> <tbody> <tr> <td>TCGA glyco 26 peps_repeatability.sky</td> <td>light</td> <td>The internal standard in the skyline file is set to be light, while the inferred internal standard is heavy. Errors happen for all the peptides.</td> </tr> </tbody> </table>	Skyline File Name	Internal Standard Type	Quality	TCGA glyco 26 peps_repeatability.sky	light	The internal standard in the skyline file is set to be light, while the inferred internal standard is heavy. Errors happen for all the peptides.	In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type , set Internal standard to be the correct type.
Skyline File Name	Internal Standard Type	Quality							
TCGA glyco 26 peps_repeatability.sky	light	The internal standard in the skyline file is set to be light, while the inferred internal standard is heavy. Errors happen for all the peptides.							
Attribute	Essential attributes have missing values.	<table border="1"> <thead> <tr> <th>Skyline File Name</th> <th>Peptide sequence with errors</th> <th>Issue reason</th> </tr> </thead> <tbody> <tr> <td>DDRpanel_052316_repeatability_03_QCTEST.sky</td> <td>LSLEFPSGYPNAPTVK, precursor charge: 2</td> <td> <ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including Replicate; Concentration; SampleGroup. </td> </tr> </tbody> </table>	Skyline File Name	Peptide sequence with errors	Issue reason	DDRpanel_052316_repeatability_03_QCTEST.sky	LSLEFPSGYPNAPTVK, precursor charge: 2	<ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including Replicate; Concentration; SampleGroup. 	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the corresponding attributes.
Skyline File Name	Peptide sequence with errors	Issue reason							
DDRpanel_052316_repeatability_03_QCTEST.sky	LSLEFPSGYPNAPTVK, precursor charge: 2	<ul style="list-style-type: none"> Essential attribute(s) has (have) missing values, including Replicate; Concentration; SampleGroup. 							
Attribute	Essential attributes are using uncontrolled terms.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the corresponding attributes.						
Attribute	Essential attributes are annotated in unqualified data type.	None	In Skyline, for the old template, View -> Document Grid -> Views -> Replicates , check the data type of the corresponding attributes; for the new template, View -> Document Grid -> Views -> Assay Repeatability Replicates , check the data type of the 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.						

<p>Area values of heavy or light Isotope</p>	<p>The number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes.</p>	<table border="1"> <thead> <tr> <th data-bbox="674 124 889 188">Skyline File Name</th> <th data-bbox="889 124 1021 188">Peptide sequence with errors</th> <th data-bbox="1021 124 1391 188">Issue reason</th> </tr> </thead> <tbody> <tr> <td data-bbox="674 188 889 448">MousePlasmaExp2B3_Refined.sky</td> <td data-bbox="889 188 1021 448">DFALQNPQVVPVPR, precursor charge: 2</td> <td data-bbox="1021 188 1391 448"> <ul style="list-style-type: none"> For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in 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 y2 (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 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). </td> </tr> </tbody> </table>	Skyline File Name	Peptide sequence with errors	Issue reason	MousePlasmaExp2B3_Refined.sky	DFALQNPQVVPVPR, precursor charge: 2	<ul style="list-style-type: none"> For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in 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 y2 (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 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). 	<p>In Skyline, for the old template, View -> Document Grid -> Views -> Replicates, check the values of the attribute of Replicate for the problematic replicate names, for example, correct the values of the attribute of Replicate for the replicate name of Day4_Std3_R01 and Day4_Std3_R03. In Skyline, for the old template, View -> Document Grid -> Views -> Replicates, check the values of the attribute of Replicate for the 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.</p>
Skyline File Name	Peptide sequence with errors	Issue reason							
MousePlasmaExp2B3_Refined.sky	DFALQNPQVVPVPR, precursor charge: 2	<ul style="list-style-type: none"> For b2 (1+): 2 heavy isotopes 2 light isotopes due to multiple values in 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 y2 (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 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). 							

Warning category	Warning message	Example case	Solution to the issue
<p>Fragment ion</p>	<p>In repeatability graph, the number of fragment ions with both heavy and light isotopes is less than 3.</p>	<p>In the repeatability graph below, the number of fragment ions is 1 < 3; the only fragment ion is: y7 (1+).</p>	<p>Check the repeatability graph to make further decisions.</p>

<p>Missing points</p>	<p>In repeatability graph, for the fragment ion, there is no point on at least one day.</p>	<p>For fragment ion y14 (2+), there are no points on day 4.</p> 	<p>Check the repeatability graph to make further decisions.</p>
<p>Missing points</p>	<p>In repeatability graph, fragment ion doesn't have three concentrations: Hi, Med, and Low on a specific day.</p>	<p>None</p>	<p>Check the repeatability graph to make further decisions.</p>
<p>Missing points</p>	<p>In repeatability graph, fragment ion has less than 3 replicates for a specific concentration on the specific day.</p>	<p>For fragment ion y5 (1+), there are less than 3 replicates in concentration(s) Hi, Lo, Med on day 1.</p> 	<p>Check the repeatability graph to make further decisions.</p>