Response Curve (Experiment 1)

Error category	Error reason	Example case	Solution to the issue
Internal standard	Internal standard is set to be none.	File: MousePlasmaV2Exp1B3_refined_2019-07-30_00-43-25.sky.zip (UVicPC_Borchers)	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard.
Internal standard	Internal standard is incorrect according to the inferred internal standard.	File: MousePlasmaV2Exp1B3_refined_2019-07-23_00-43-25.sky.zip (UVicPC_Borchers)	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	File: DDRpanel_052316_curves_05_QCTEST.sky.zip (Chenwei_dataset) Peptide sequence: LELAEQK File: 20160309_MouseV2B1_refined_2018-07-03_14-57-32.sky (UVicPC_Borchers)	1. 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. 2. 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, fragment ions y6 and b4 have no data, delete them.
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.	File: MousePlasmaV2Exp1B3_refined_2018-12-21_14-36-25.sky.zip (UVicPC_Borchers)	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.
Concentration	More than one concentration levels are needed.	None	In Skyline, for the old template, **View** -> **Document Grid -> Views -> Replicates**, check the attribute of Concentration; for the new 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, fragmention, replicate, concentration, samplegroup, isspike and isotopelabel.	Peptide sequence: ETPAASEAPSSAAK File: 20160720_MousePlasmaExp1Batch5_refined_2017-12-11_11-50-02.sky.zip (UVicPC_Borchers)	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.
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 reason	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.	Peptide sequence: LNT[+80]SDFQK File: E126_MP_EXP1_RevCurves_20141215_UPL OAD DOC_2015-06-08_14-46-49.sky.zip (WUSTL_Townsend)	Check the response curve graph to make further decision.
Bad linear regression fitting	The coefficient of variance of slopes of fragment ions is larger than 0.5.	Peptide sequence: VYPLINR File: E126_MP_EXP1_RevCurves_20141215_UPL OAD DOC_2015-06-08_14-46-49.sky.zip (WUSTL_Townsend)	Check the response curve graph to make further decision.
Bad linear regression fitting	The slopes of the fragments ions are 0.	None	Check the response curve graph to make further decision.
Bad linear regression fitting	The fit of the linear regression model is poor due to the low R^2 (coefficient of determination) or large p value of F-test in the process of linear regression.	Peptide sequence: HEDILSILTAVNDDVSR File: E126_MP_EXP1_RevCurves_20141215_UPL OAD DOC_2015-06-08_14-46-49.sky.zip (WUSTL_Townsend)	Check the response curve graph to make further decision.

Repeatability (Experiment 2)

Error category	Error reason	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.	File: TCGA glyco 26 peps_repeatability_2019-08-12_14-35-45.sky.zip (JHU_DChan_HZhang_ZZhang)	In Skyline, **settings** -> **Peptide Settings** -> **Modifications** -> **Internal standard type**, set Internal standard to be the correct type.
Attribute	Essential attributes have missing values.	File: DDRpanel_052316_repeatability_03_QCTEST.sky.zip (Chenwei_dataset)	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 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.	File: MousePlasmaExp2B3_Refined_2019-06-11_16-46-36.sky.zip (UVicPC_Borchers)	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.
Area values of heavy or light Isotope	Number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes.	Peptide sequence: DFALQNPGVVPR File: MousePlasmaExp2B3_Refined_2017-12-11_15-09-36.sky.zip (UVicPC_Borchers)	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; 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 category	Warning reason	Example case	Solution to the issue
Fragment ion	In repeatability graph, the number of fragment ions with both heavy and light isotopes is less than 3.	Peptide sequence: LLIIDSNLGVQDVENLK File: E130_MP_EXP2_P7_20150105_UPLOAD DOC_2015-06-08_15-23-30.sky.zip (WUSTL_Townsend)	Check the repeatability graph to make further decision.

Missing points	In repeatability graph, fragment ion has missing points on the specific day.	Peptide sequence: EGITDAATMK File: 082618_iMRM_mAb-Plex2_Exp2_Repeatability_upload_v4_replicates_2018-08-28_09-09-50.sky.zip (Koomen)	Check the repeatability graph to make further decision.
Missing points	In repeatability graph, fragment ion doesn't have three concentrations: Hi, Med, and Low on the specific day.	None	Check the repeatability graph to make further decision.
Missing points	In repeatability graph, fragment ion has less than 3 replicates for a specific concentration on the specific day.	Peptide sequence: GLDARPEVTR File: 082618_iMRM_mAb-Plex2_Exp2_Repeatability_upload_v4_replicates_2018-08-28_09-09-50.sky.zip (Koomen)	Check the repeatability graph to make further decision.