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 Quality Type | Internal standard type, set Internal standard. |
| | | MousePlasmaVZExp183_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 | 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. | 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. | type. |
| Attribute | Essential attributes have | Peptide sequence with | 1. In Skyline, for the old template, View -> Document Grid -> |
| | missing values. | Skyline File Name errors issue reason | Views -> Replicates, check the corresponding attributes; for the |
| | | Essential attribute(s) has (have) missing values, including | new template, View -> Document Grid -> Views -> Assay Curve |
| | | ReplicateName; Replicate SampleGroup. DDRpanel_052316_curves_05_QCTEST.sky DLSHIGDAVVISC[+57]AK[+114]DGVK | Replicates , check the corresponding attributes. |
| | | - Issential activities of the state of the s | 2. If the corresponding attributes are normal in step 1, in Skyline, |
| | | 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 | Peptide | In Skyline, for the old template, View -> Document Grid -> Views |
| peptide | concentration of the | Skyline File Name sequence with errors Issue reason | -> Replicates, check the attribute of IS Spike, then View -> |
| concentration | internal standard peptide | The annotation of the concentration of the internal standard peptide has issues. Please the the doctor of IS Spike or check the annotation of IS Spike or check the annotation of IS Spike or check the annotation. | |
| | has issues. | PeptideConcentrationIS. | 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, peptidemodifieds equence, precursorcharge, product charge, fragmention, replicate, concentration, samplegroup, isspike and | 20160720_MousePlasmaExplBatch5_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 | isotopelabel. 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 1.5- Transition 2 SUM 2 2 y4.1 2 2 y5.1 Theoretical Concentration (fmol/uL) | Check the response curve graph to make further decisions. |
| Bad linear | The coefficient of variance | Analyte: O95819.VYPLINR | Check the slopes of the fragment ions in the response curve graph |
| regression fit | of slopes of the fragment ions is larger than 0.5. | Transition 2 b5 1 2 SUM 2 2 y4 1 2 2 y5 1 10 10 10 Theoretical Concentration (fmol/uL) | to make further decisions. |

| Bad linear | The fit of the linear | Check the response curve graph to make further decisions. |
|--------------------|--------------------------------|---|
| regression fitting | regression model is poor | Analyte: Q92851.HEDILSILTAVNDDVSR |
| | due to $R^2 < 0.5$ or p > 0.05 | 30- Transition |
| | in the significance test for | ◆ 3.57.1 ◆ 3.5UM. |
| | linear regression. | * 3y6.1 * 3y7.1 |
| | | . <u>Q</u> 20- |
| | | Deak Area Ratio |
| | | × Are |
| | | D D 10- |
| | | |
| | | |
| | | |
| | | 0.0 2.5 5.0 7.5 10.0 |
| | | Theoretical Concentration (fmol/uL) |
| | | |

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 light, while the inferred internal standard is heavy. Errors happen for 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 reason • Essential | -> Replicates, check the corresponding attributes; for the new |
| | | attribute(s) has (have) missing | template, View -> Document Grid -> Views -> Assay |
| | | DDRpanel_052316_repeatability_03_QCTEST.sky LSLEFPSGYPYNAPTVK, precursor including Replicate; Concentration; SampleGroup. | 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 | | | |
|----------------|---|-------------------------------|---|--|
| heavy or light | isotopes or heavy isotopes | Skyline File Name | Peptide sequence with errors | Issue reason |
| Isotope | is not one due to wrongly annotated values in attributes. | MousePlasmaExp2B3_Refined.sky | DFALQNPGVVPR, precursor charge: 2 | • 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 isotope 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y2 (1+): 2 heavy isotope 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y3 (1+): 2 heavy isotope 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R03). For y6 (1+): 2 heavy isotope 2 light isotopes due to multiple values in attributes: replicate_name (Day4_Std3_R01 Day4_Std3_R01 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 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.

| Warning | Warning message | Example case | Solution to the issue |
|-----------------------|--|--|--|
| category Fragment ion | In repeatability graph, the number of fragment ions with both heavy and light isotopes is less than 3. | In the repeatability graph below, the number of fragment ions is 1 < 3; the only fragment ion is: y7 (1+). Steadydout/traterogyssr | Check the repeatability graph to make further decisions. |
| Missing points | In repeatability graph, for the fragment ion, there is no point on at least one day. | For fragment ion y14 (2+), there are no points on day 4. SLEATDSAFDNPDYWHSR y14 (2+) Property of the part of the | Check the repeatability graph to make further decisions. |
| Missing points | In repeatability graph, fragment ion doesn't have three concentrations: Hi, Med, and Low on a specific | None | Check the repeatability graph to make further decisions. |

| | day. | | |
|----------------------------|--|------|--|
| Missing points | In repeatability graph, fragment ion has less than 3 replicates for a specific concentration on the specific day. | | Check the repeatability graph to make further decisions. |
| Bad distribution of points | In repeatability graph, for the fragment ion, the intra- assay, inter-assay or total coefficient of variance is larger than the threshold of 0.2. | None | Check the repeatability graph to make further decisions. |

Selectivity (Experiment 3)

| 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 | In Skyline, settings -> Peptide Settings -> Modifications -> Internal standard type, set Internal standard. |
| Internal standard | Internal standard is incorrect. | Skyline File Name Standard Type Batch1_Exp3_20160519_M1.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. |
| Attribute | Essential attributes have missing values. | None | In Skyline, View -> Document Grid -> Views -> Assay Selectivity Replicates, check the corresponding attributes. |
| Attribute | Essential attributes are annotated in unqualified data type. | None | In Skyline, View -> Document Grid -> Views -> Assay Selectivity 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. |
| Concentration | More than one concentration levels are needed. | None | In Skyline, View -> Document Grid -> Views -> Assay Selectivity Replicates, check the attribute of Analyte Concentration. |
| 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. | Skyline File Name Peptide sequence with errors For ys (1*); 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate, analyte concentration or sample group. For ys (1*): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate, analyte concentration or sample group. For ys (1*): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate, analyte concentration or sample group. For ys (1*): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes; replicate, analyte concentration or sample group. | In Skyline, View -> Document Grid -> Views -> Assay Selectivity Replicates, check the values of the attribute of Replicate Number, Analyte Concentration and Exp3 SampleGroup. |

| 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 | None | |
| | due to R ² < 0.5 for some | | |
| | fragment ions in the | | |
| | significance test for linear | | |
| | regression. | | |

Stability (Experiment 4)

| 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 | 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 CPTAC_TemplateDoc_Exp4_Stability_DRAFT02_2020- 01-09_10-45-35.sky.zip Peptide sequence with errors LDQGGILPGSNR, precursor charge: 2 • Essential attribute(s) has (have) missing values, including ReplicateNumber. | I. In Skyline, View -> Document Grid -> Views -> Assay Stability Replicates, check the corresponding attributes. |
| Attribute | Essential attributes are annotated in unqualified data type. | None | In Skyline, View -> Document Grid -> Views -> Assay Stability Replicates, check the data types of corresponding attributes. |
| Attribute | Incorrect annotations in the attribute of Exp4 Sample Group or Freeze Thaw Cycles. | None | In Skyline, View -> Document Grid -> Views -> Assay Stability Replicates, check 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 | The number of light isotopes or heavy isotopes is not one due to wrongly annotated values in attributes. | Peptide sequence with errors - For y5 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y6 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y6 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y9 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y9 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y9 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. For y9 (1+): 2 heavy isotopes 2 light isotopes due to wrongly annotated values in attributes: replicate number, time or sample group. | In Skyline, View -> Document Grid -> Views -> Assay Stability Replicates, check the values of the attribute of Replicate Number, Time, Time Units and Exp4 SampleGroup. |

| 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 P-value from ANOVA: 0.94 [algos 0.07 - 0.08 - 0.07 - 0.08 - 0.07 - 0.08 | 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 Condition Control Friza Autosampler Frizaen Replicate 1 2 3 4 4 5 | 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 | | |
| | 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 Standard Type CPTAC_TemplateDoc_Esp5_Reproducibility_DRAFT01_2020- 03-26_99-58-55.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 | 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. | | |