Intact Mass Screening

Biologics Explorer Software Guidelines

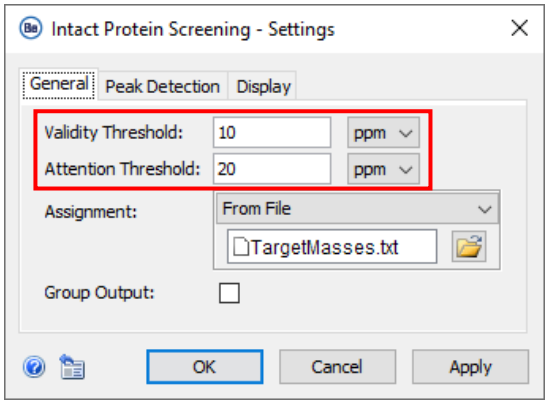
Powered by Genedata Expressionist®

Genedata solutions in silico

SCIEX The Power of Precision

Intact Mass Screening Workflow: Overview

- This workflow provides quick deconvolution for high-throughput screening of large batches of samples.
- It can verify the presence, or absence, of target masses within specified limits of mass confidence (ppm or Da).
- The visual summary table identifies each sample as:
  - Valid (✓): The calculated mass is below the Validity Threshold.
  - Critical (!!): The calculated mass is between the Validity and Attention Threshold.
  - Invalid (✗): The calculated mass is above the Attention Threshold.

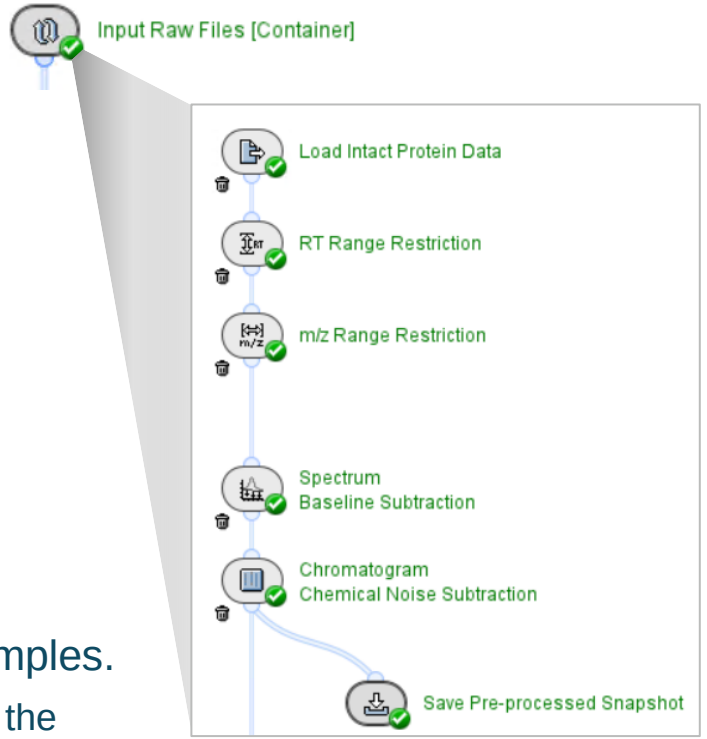


Zoomed Range 3

Name	Expected Mass	Detected Mass	Delta [Da]	Delta [ppm]	Valid
Remicade_IdeS_reduced	25647.51	25647.35	-0.17	-6.0	✓
Rituxan_IdeS_reduced	25328.19	25327.79	-0.41	-16.0	✗
Herceptin_IdeS_TCEP	25383.31	25383.14	-0.16	-6.0	✓
Humira_IdeS_TCEP	25458.33	25457.95	-0.37	-15.0	!!
NIST_500ngOC_IdeS_Red_01	25688.91	25688.72	-0.19	-7.0	✓

Intact Mass Screening Workflow: Overview

- To reduce the computational memory used for high-throughput screening:
  - The *Input Raw Files [Container]* uses an iterative process to pre-process each sample in the batch independently.
  - **Trash** is activated in the container so that intermediate results are deleted as soon as they are passed to the subsequent activity node to save memory.
  - Results from each pre-processed sample can be saved as a snapshot (sbf) file that can be used in other intact mass analysis workflows for further investigation, if required.



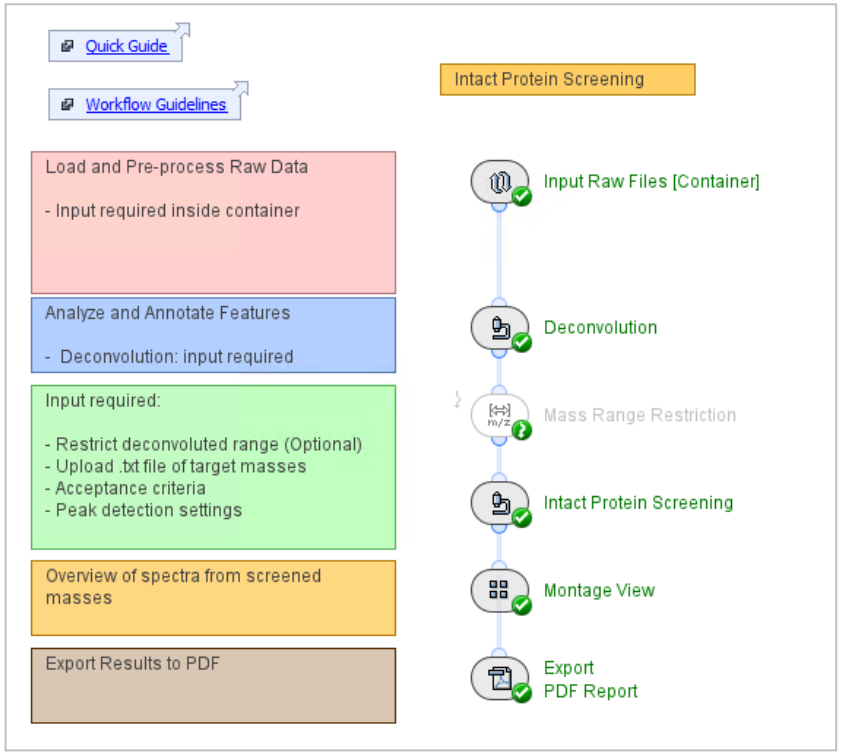
- The *Montage View* activity node can display up to 200 samples.
  - If more than 200 samples are analyzed together, then activate the **Bypass** icon on the *Montage View* activity node.

Intact Mass Screening Workflow: Conditions and Behavior

- For each sample, more than one mass can be searched. However, certain conditions should be met in the submitted samples:
  - Samples must belong to the same molecule type. For example, all samples should be either intact proteins, subunits, or fragments.
  - Samples should have consistent chromatography, the same number of components, and similar expected deconvolution ranges.
  - Samples must have the same number of either **Full** or **Zoomed RT Ranges**.

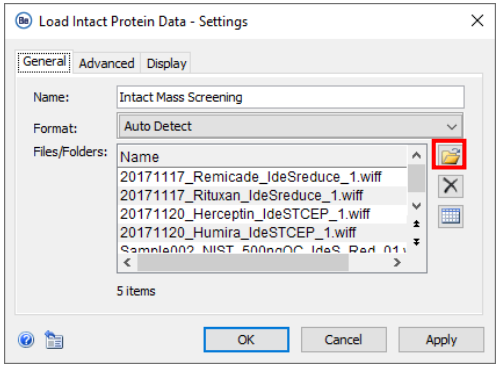
- The workflow is designed to analyze data as follows:
  - The highest peak in each deconvoluted RT range (**Full** or **Zoomed RT Range**) of each sample is detected independently and assigned to a single match based on the list of masses.
  - Results do not include annotation.
  - The values of the detected masses are reported.

Overview of the Intact Mass Screening Workflow

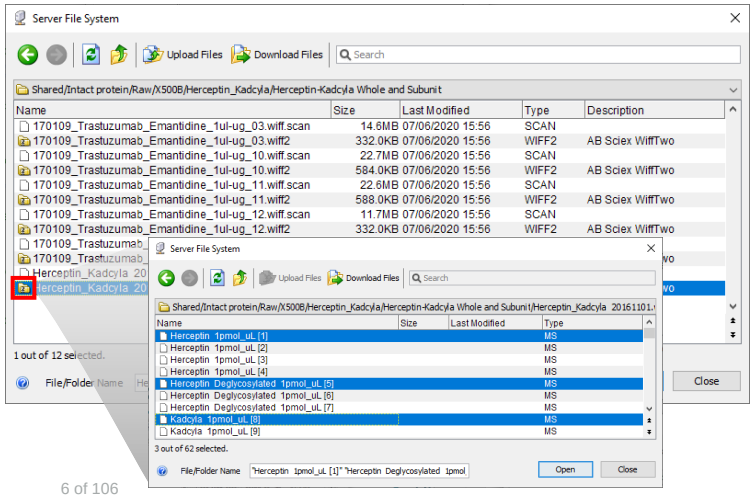


Intact_MassScreening_Be4.0

Load Intact Protein Data



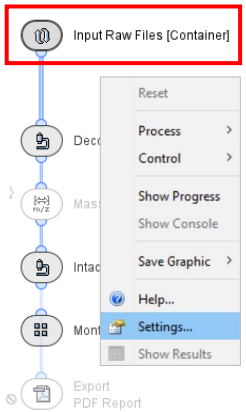
- To upload raw data files, click the folder icon .
  - Select container files with the format wiff or wiff2.
    - If data was acquired with the ZenoTOF 7600 system, select only the wiff2 format.
    - Do not select the auxiliary files with the same name.



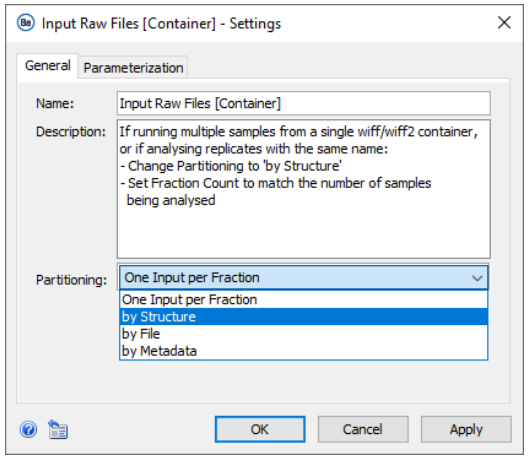
  - To select samples in a wiff or wiff2 container file:
    - Double-click the wiff or wiff2 container to see the sample files.
    - Select the samples to upload from the list of sample files.
      - Note: For more information, refer to the page: *Input Raw Files [Container]*.

Input Raw Files [Container]

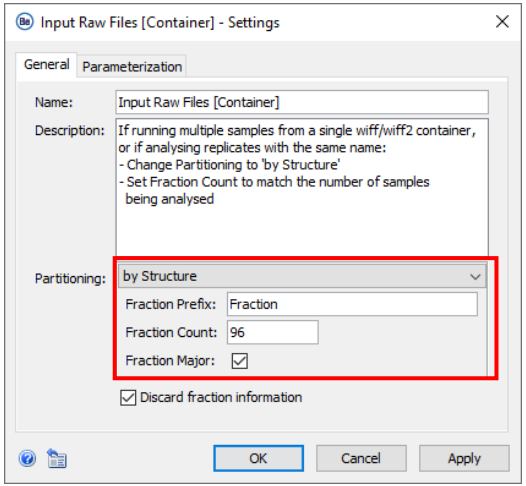
- The settings of the *Input Raw Files [Container]* must be changed to:
  - Analyze replicate samples that have the same file name.
  - Load multiple different samples from in a single wiff or wiff2 container.



(1) Right-click on *Input Raw Files [Container]* to access Settings.



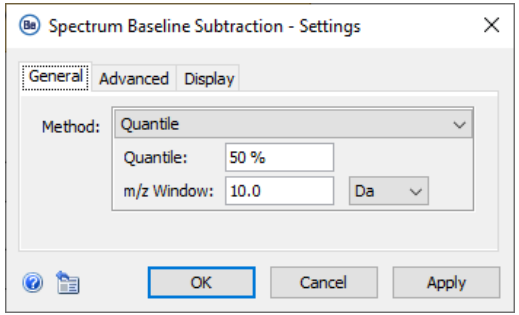
(2) Change **Partitioning** to **by Structure**.



(3) Specify the number of samples loaded as the **Fraction Count**.

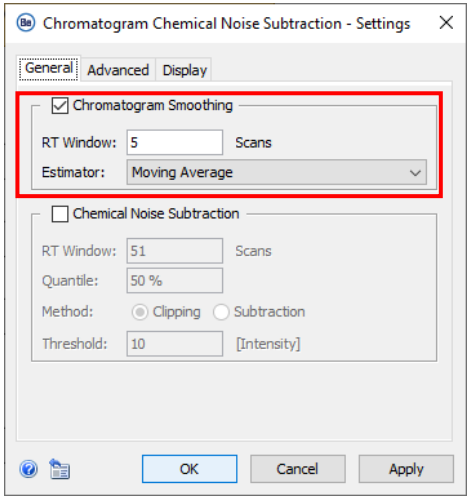
Spectrum Baseline Subtraction

- *Spectrum Baseline Subtraction* removes background noise and decreases the intensity of satellite peaks in the deconvoluted data.



- **Quantile** subtraction affects all signals:
  - It requires little or no smoothing afterwards.
  - It is much faster than **Penalized Least Squares** when used with high resolution data.
  - It should be used with care for the analysis of intact proteins to prevent removal of meaningful signals.
- **Penalized Least Squares** subtraction has an effect on low intensity signals only.

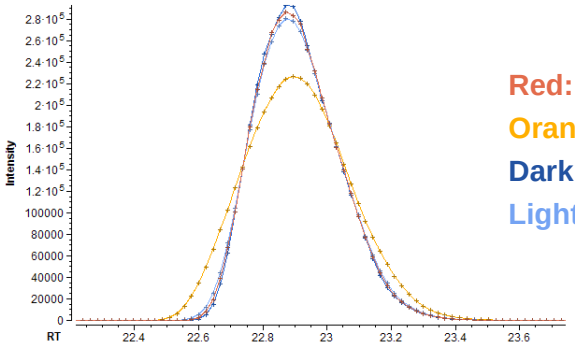
Chromatogram Chemical Noise Subtraction: Smoothing



Chromatogram Smoothing is used to improve the RT profile of peaks.

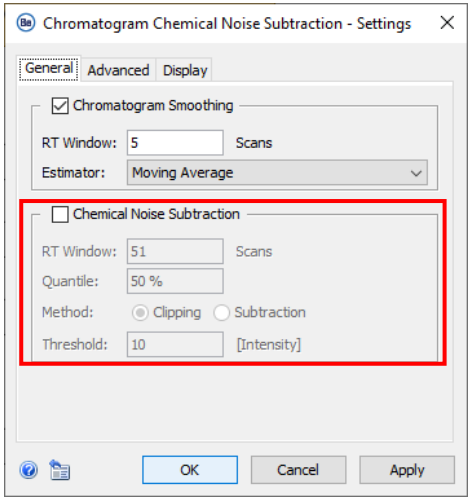
– Use **Chromatogram Smoothing** after **Penalized Least Squares** (in *Spectrum Baseline Subtraction*), especially if a high **Eagerness** value was used.

- **Estimator:**
  - **Moving Average** uses the mean intensity of data points in the **RT Window** to add more data points. High values cause peak widths to increase, but peak volume is conserved.
  - **Binomial** is an iterative form of **Moving Average** that has less effect on peak widths at high scan values.



Red: Moving Average (5 scans)
Orange: Moving Average (15 scans)
Dark Blue: Binomial (5 scans)
Light Blue: Binomial (15 scans)

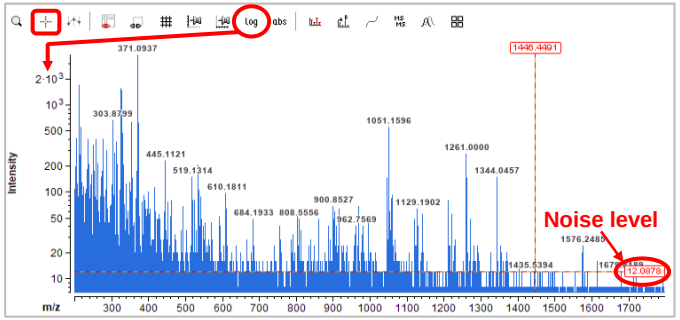
Chromatogram Chemical Noise Subtraction: Threshold



Chemical Noise Subtraction can help to:

- Reduce the broad or long-tailing peaks often observed with native data.
- Suppress satellite peaks and improve peak detection with TRD.

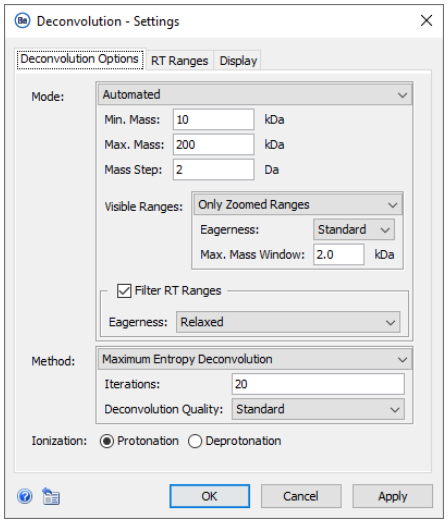
- To decrease the amount of noise removal (keep more signal):
  - Decrease the **Quantile**.
  - Increase the **RT Window**.
- If the noise level is significantly different from the **Threshold** value pre-set in *Chromatogram Chemical Noise Subtraction*, then change this setting.



- To measure the noise level and specify an appropriate **Threshold** intensity value:
  1. Drag the mass spectrum intensity axis until the noise level can be seen, or use the icon in the tool bar to change the axis from the linear to the logarithmic scale.
  2. Use the crosshair tool to measure the intensity of the noise level.

Deconvolution

Deconvolution

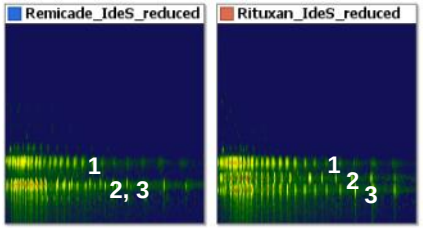


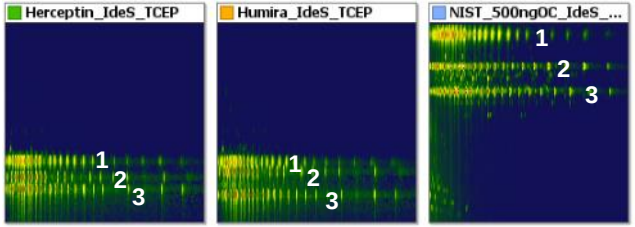
Specify the relevant mass range and visualization options for the deconvoluted spectra.

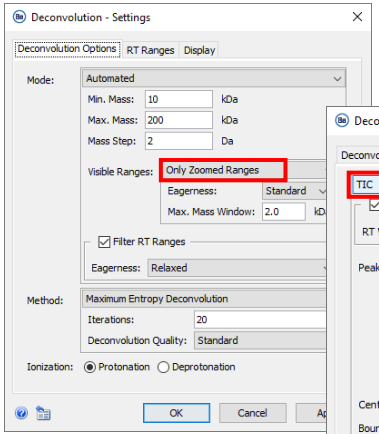
- **Mass range**:
  - To analyze multiple species in the same data file, use a wide mass range to reduce prominent harmonics peaks.
  - To focus on a single species, use a narrow mass range.
- **Visible Ranges** controls how the results are displayed.
  - Use **Only Zoomed Ranges** if multiple components are detected in the same RT range.
- Set a **Mass Step** value that results in the same number of data points across peaks before and after deconvolution.
  - 0.1 Da - 0.2 Da for isotopically resolved data.
  - 1 Da for subunits (lower-resolution data).
  - 2 Da for intact proteins (lower-resolution data).
  - 3 Da if fewer datapoints are required.

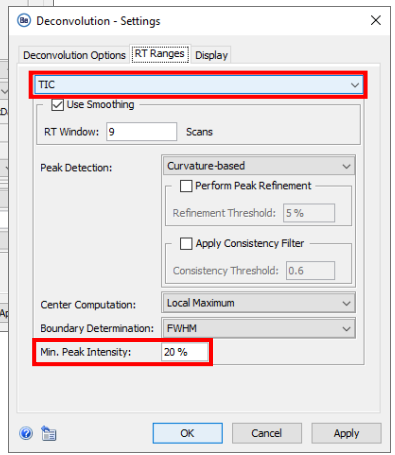
Deconvolution: Complex Datasets

- The template workflow represents a highly complex screening application:
  - There are three target components (mAb fragments), from different mAbs.
  - Chromatography is inconsistent.
  - The Fc/2 glycoforms have similar intensity, and the intensity order changes across the samples.
  - Data is from both wiff and wiff2 files.



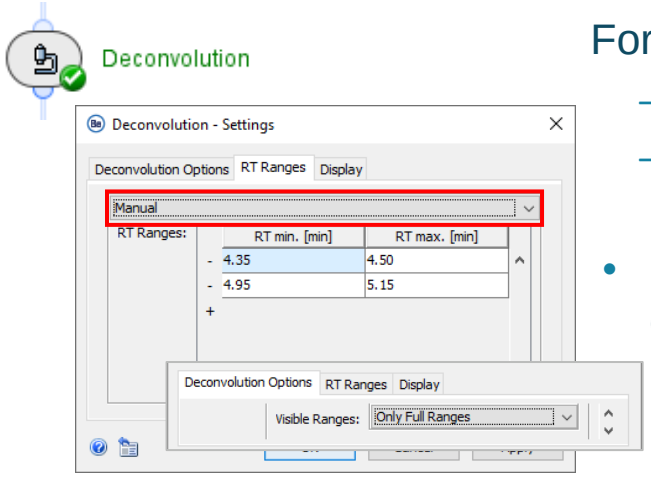


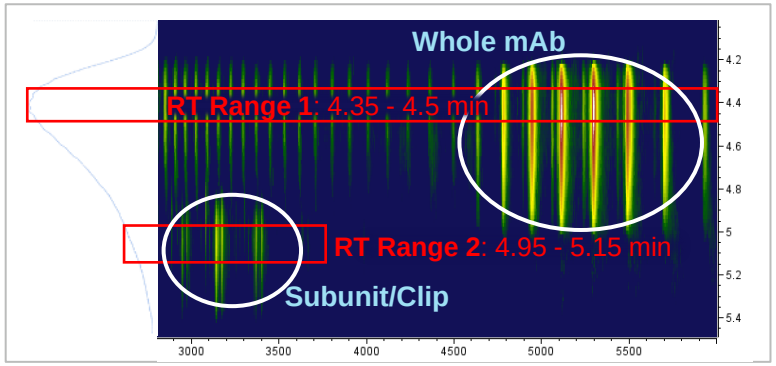




- To overcome the potential challenges with this type of dataset:
  - Use **TIC** to automatically identify the specific RT ranges for each sample.
  - Use a **Min. Peak Intensity** of 20% (or higher) to limit the number of deconvoluted RT ranges to those related to the target fragments.
  - Use **Only Zoomed Ranges** as these are expected to be the same for all screened samples in the same batch, if they are the same type of molecule.

Deconvolution: RT Ranges





For the use case where a screening workflow is used to analyze:

- Main target component: Whole mAb (always expected).
- Known side product: Misconnected subunit that may be present or absent.

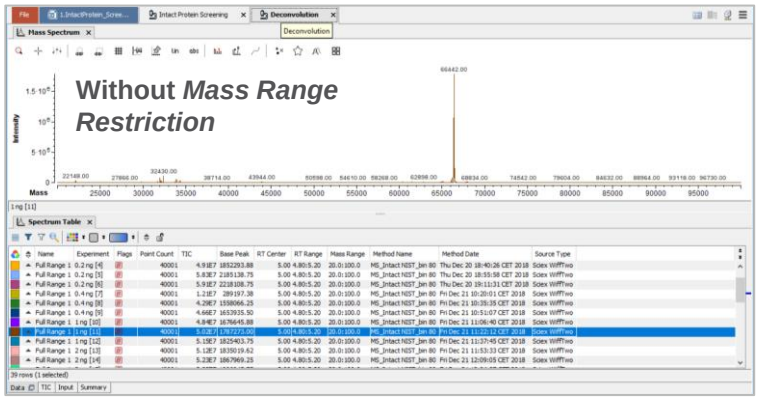
- Use of the **TIC** to identify the RT ranges would result in variable numbers of RT ranges per sample. This inhibits the screening workflow.

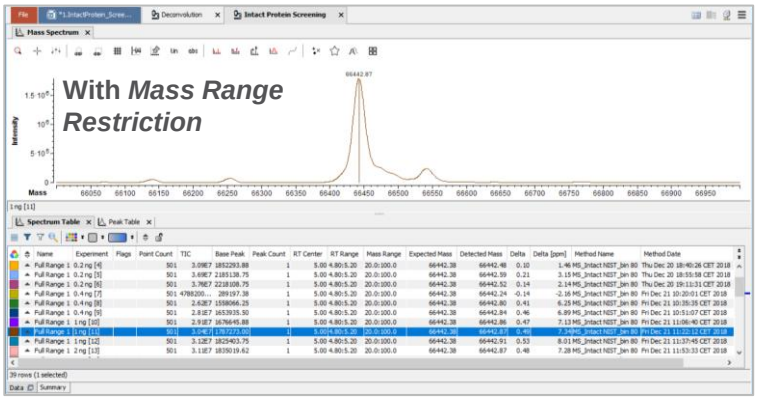
The optimal settings for **RT Ranges** when components are not always present are:

- Use **Manual** to specify the RT ranges for each component (small RT windows focused on the apex of the elution profiles).
- Set **Visible Ranges** to **Only Full Ranges**.
- The workflow will complete, if:
  - The chromatography is consistent over the sample batch.
  - There is some separation between the components.

Mass Range Restriction

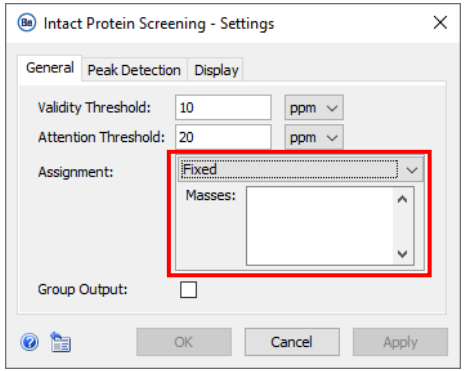
- This activity node is optional. To use *Mass Range Restriction*, deactivate the **Bypass** icon.
- *Mass Range Restriction* can be used to restrict how the deconvoluted spectra of specific target mass is shown.
  - The restriction continues to be applied when **Only Full Ranges** is used.
  - This activity node can be used as an alternative to **Only Zoomed Ranges**.



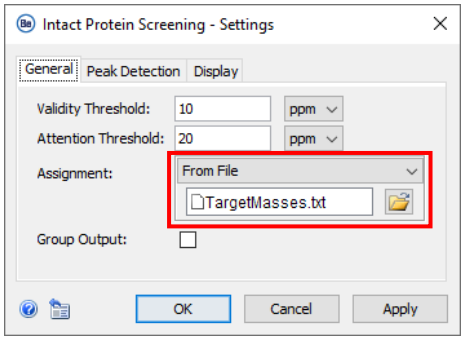


To Define Target Masses for *Intact Protein Screening*

- Specify the masses of interest for screening:



- If the same masses are expected across all samples, then change **Assignment** to **Fixed** and type the values in the **Masses** section.



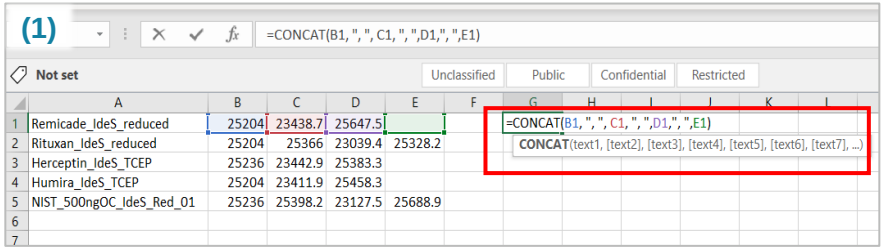
- If different masses are expected across the samples, then change **Assignment** to **From File** and upload a txt file that contains the target masses for each sample.

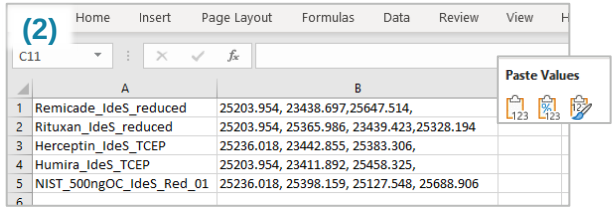
- There is no need to change the default **Peak Detection** settings.

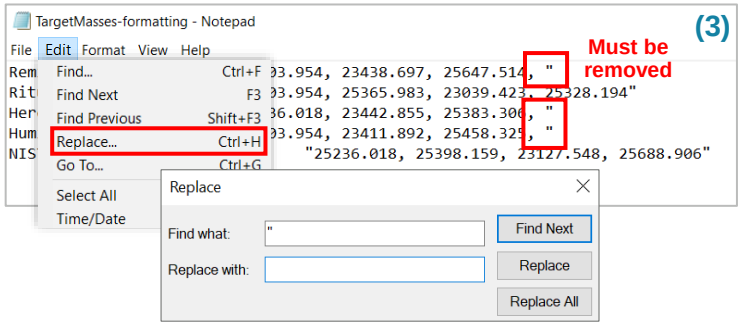
Intact Protein Screening: Target Masses File Format

To create a txt file for screening:

1. Create a table that contains sample names and target masses. Do not include table headers.
   - Use the CONCATENATE function in Excel to combine columns that contain the targeted masses into one column.
   - Notice the format **“, “**.
2. Remove formulas from the table.
   - Copy the concatenated column and select **Paste Values**.
   - Delete the original columns to produce a two-column table.
   - Save the spreadsheet as txt file.
3. Open the txt file and remove any additional, unrequired characters.
   - Manually remove duplicate commas or commas at the end of a sequence of masses.
   - If **“** is present: Use the Replace tool to replace “ with a space. Then save the txt file.





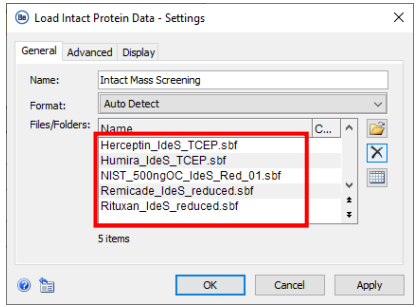


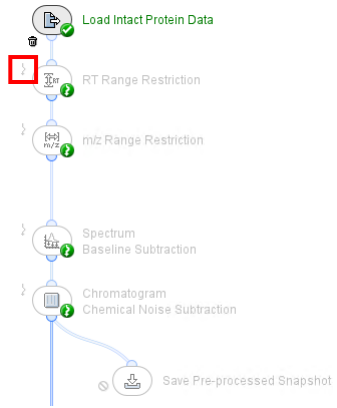
Export Intermediate Results for Further Analysis

- The *Save Snapshot* activity node stores intermediate results after pre-processing.
  - An individual sbf file is saved for each sample processed in the workflow.
  - The sbf file contains the properties of the processed data that are required to continue analysis from that point in the workflow.

- To use a *Save Snapshot* activity node:
  - Deactivate the **Block** icon.
  - Select or add the folders where the sbf files will be stored.

- To use intermediate results from *Save Pre-processed Snapshot*:
  1. Select the sbf file to import into *Load Intact Protein Data*.
  2. Deactivate the **Trash** icon, and then activate the **Bypass** icon on the activity nodes that are before the Snapshot was saved.

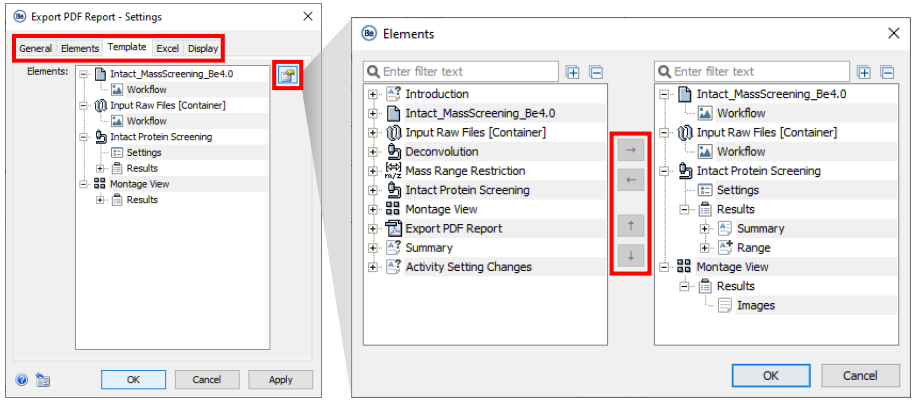




Export PDF Report

- The exported PDF Report includes:
  - A PDF document.
  - An Excel file with spectral information from deconvolution.
  - An embedded workflow (xml file) that includes all of the settings.
    - To open the xml file, drag the saved PDF Report into the workflow home page in the Biologics Explorer software.
    - Note: For more information, refer to the document: Biologics Explorer Quick Guide.

- **General** tab: Specify the name and saved location of the exported report.
- **Template** tab: Use the **Edit Selection** icon to specify the **Elements** to be included in the report.
  - Select only columns of interest in reported tables. The layout of the tables is controlled by the number of columns.
- **Excel** tab: Use the **Edit Selection** icon to specify the **Tables** to be included in the report.
  - All columns in a selected table are reported.



SCIEX

The Power of Precision

For more information, visit the SCIEX website at sciex.com, or contact us in one of the following ways:

sciex.com/contact-us
sciex.com/request-support

Trademarks / Licensing

The SCIEX clinical diagnostic portfolio is For In Vitro Diagnostic Use. Rx Only. Product(s) not available in all countries. For information on availability, please contact your local sales representative or refer to https://sciex.com/diagnostics. All other products are For Research Use Only. Not for use in Diagnostic Procedures.